COUNTWAY LIBRARY
HC 6E01

BOSTON
MEDICAL LIBRARY
8 THE FENWAY

THE
ANATOMICAL RECORD

MANAGING EDITOR

Charles M. Goss
Louisiana State University

ASSOCIATE EDITORS

William U. Gardner
Yale University

William W. Greulich
Stanford University

Normand L. Hoerr
Western Reserve University

Thomas E. Hunt
University of Alabama

Gordon H. Scott
Wayne University

Raymund L. Zwemer
Library of Congress

VOLUME 110

MAY, JUNE, JULY, AUGUST

1951

PUBLISHED BY

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

PHILADELPHIA, PA.

CATALOGUED
MAR 26 1952
G.G.Q.

BOSTON
MAR 26 1952
MEDICAL LIBRARY

7334
6-3

CONTENTS

No. 1 MAY 1951

Otto F. Kampmeier and Emil W. Hospodar. Mounting of stained serial slices of the brain as wet specimens in transparent plastic. Three figures 1

J. D. Feldman. Endocrine control of lymphoid tissue. Eighteen figures 17

Wilhelm Harkmark and Knut Graham. A method for opening and closing eggs with an experimental demonstration of its advantages. Eight figures 41

Arthur H. Stein, Jr. Variations of the tendons of insertion of the abductor pollicis longus and the extensor pollicis brevis. Five figures 49

J. F. A. McManus, C. H. Lupton, Jr., and L. S. Graham, Jr. The demonstration of the intercapillary space of the human renal glomerulus. Two figures 57

Thaddeus H. Doggett. The capillary system of the dog's spleen. Ten figures 65

Maury Massler and Isaac Schour. The growth pattern of the cranial vault in the albino rat as measured by vital staining with alizarine red "S." Four figures 83

Newton B. Everett and Robert J. Johnson. A physiological and anatomical study of the closure of the ductus arteriosus in the dog. Five figures ... 103

Charles A. Reed. Pericardio-peritoneal herniae in mammals, with description of a case in the domestic cat. Two figures 113

No. 2 JUNE 1951

R. M. Wotton and P. A. Village. The transfer function of certain cells in the wall of the Graafian follicle as revealed by their reaction to previously stained fat in the cat. Three figures 121

George H. Paff, Armand W. Angulo and F. C. Eksterowicz. The zone of mineralization and the action of alizarin in preventing its appearance in bones growing in tissue culture. Four figures 129

R. M. Mulligan and K. C. Francis. Weights of thyroid and parathyroid glands of normal male dogs. Three figures 139

C. E. Lumsden with the technical assistance of Mary F. Orr and Dorothy Robbins and photography by Curtis Hoskins and George Lefeber. Aspects of neurite outgrowth in tissue culture. Ten figures ... 145

Alden B. Dawson. Histogenetic interrelationships of oocytes and follicle cells. A possible explanation of the mode of origin of certain polyovular follicles in the immature rat. Thirteen figures 181

Surindar Nath Bhaskar, Isaac Schour, Edwin Carleton MacDowell and Joseph Peter Weinmann. The skull and the dentition of screw tail mice. Thirty-one figures 199

H. Stanley Bennett. The demonstration of thiol groups in certain tissues by means of a new colored sulfhydrol reagent. Five figures 231

Arthur J. Gatz and O. Boyd Houchin. Studies on the heart of vitamin E deficient rabbits. Thirteen figures 249

R. M. Wotton and A. Martin, Jr. The reaction of the lung in the cat toward oil droplets in the circulation. Five figures 267

No. 3 JULY 1951

Helen Kutuzov and Harry Sicher. The filiform and the conical papillae of the tongue in the white rat. Six figures 275

Kōsuke Ōmura and Bunsuke Osogoe. Saponin induced colonization of the bone marrow elements in foreign organs in rabbits. Eleven figures 289

Charles Schwartz, Earl B. Scott and Ralph L. Ferguson. Histopathology of amino acid deficiencies. I. Phenylalanine. Twenty-seven figures 313

D. Louise Odor and Richard J. Blandau. Observations on the formation of the second polar body in the rat ovum. Sixteen figures 329

Homer B. Latimer. The weight of the eyeballs in the guinea pig 349

G. B. Wislocki, D. W. Fawcett and E. W. Dempsey. Staining of stratified squamous epithelium of mucous membranes and skin of man and monkey by the periodic acid-Schiff method. Thirteen figures 359

AMERICAN ASSOCIATION OF ANATOMISTS. Sixty-fourth annual meeting, Wayne University School of Medicine, Detroit, Michigan, March 21, 22, and 23, 1951. Proceedings, officers, and list of members 377

No. 4 AUGUST 1951

WILLIAM H. LEE. The glycogen content of various tissues of the chick embryo. One figure 465

A. V. NALBANDOV, R. K. MEYER AND W. H. MCSHAN. The role of a third gonadotrophic hormone in the mechanism of androgen secretion in chicken testes. Thirteen figures 475

HABEEB BACCHUS. Cytological distribution of cholesterol and ascorbic acid in the adrenal cortex of the rat exposed to cold. Six figures 495

DANIEL C. PEASE AND RICHARD F. BAKER. Electron microscopy of nervous tissue 505

DANIEL C. PEASE. Triple embedding for the ultrathin sectioning required by electron microscopy. Three figures 531

DANIEL C. PEASE. Electron microscopy of sectioned teeth. Five figures 539

JOSEPH H. GARDNER AND HARRY E. RAYBUCK. Cleavage line patterns of the cat. Three figures 549

CLARA EDDY HAMILTON. Evidences of cyclic reproductive phenomena in the rabbit. Eight figures 557

PAUL B. SAWIN AND DORCAS D. CRARY. Morphogenetic studies of the rabbit. X. Racial variations in the gall bladder. Two figures 573

MOUNTING OF STAINED SERIAL SLICES OF THE BRAIN AS WET SPECIMENS IN TRANSPARENT PLASTIC

OTTO F. KAMPMEIER AND EMIL W. HOSPODAR
Department of Anatomy, College of Medicine, University of Illinois, Chicago

THREE FIGURES

INTRODUCTION

In 1947 and 1948 when Kampmeier and Haviland described and demonstrated their methods of embedding and mounting in transparent plastics (especially in the synthetic resin "Castolite") different kinds of anatomical museum preparations — wet specimens, dehydrated and cleared specimens, dry specimens, corrosion specimens, stained specimens, injected specimens, embryological specimens, etc. — there were also exhibited among them slices of the human brain (fig. 7, p. 224, Anat. Rec., vol. 100, 1948) dyed according to Landau-Sincke's Berlin-blue method. As was pointed out at that time, the slices, though surface-dried before embedding, showed a disturbing (even if transparent) fluid zone or halo around them after embedding because of the heat engendered during polymerization of the resin, which drove the water to their surface. These preparations became a disappointment also for another reason, for after some months their color faded or diffused unevenly from the gray to the white substance. Yet they were almost the first to arouse the greatest interest. Neurologists, with varied objectives, were unamimous in demanding that improved methods of staining and of plastic embedding of gross preparations of the brain be devised. Because of the burden of other duties, the senior author could not continue his experiments until a year ago. Then, with the

THE ANATOMICAL RECORD, VOL. 110, NO. 1
MAY 1951

help of the departmental technicians, Emil Hospodar and Ralph Bouldin, many trials were made of which the positive results are set forth in the present paper. The specimens herein illustrated (figs. 1–3) are representative of those prepared about a year ago. None of them having undergone any visible deterioration or change since that time, it may be supposed that they are relatively permanent.

The primary incentive in this as in the earlier work (Kampmeier, '33, '48 and '50) was to add satisfactory specimens to

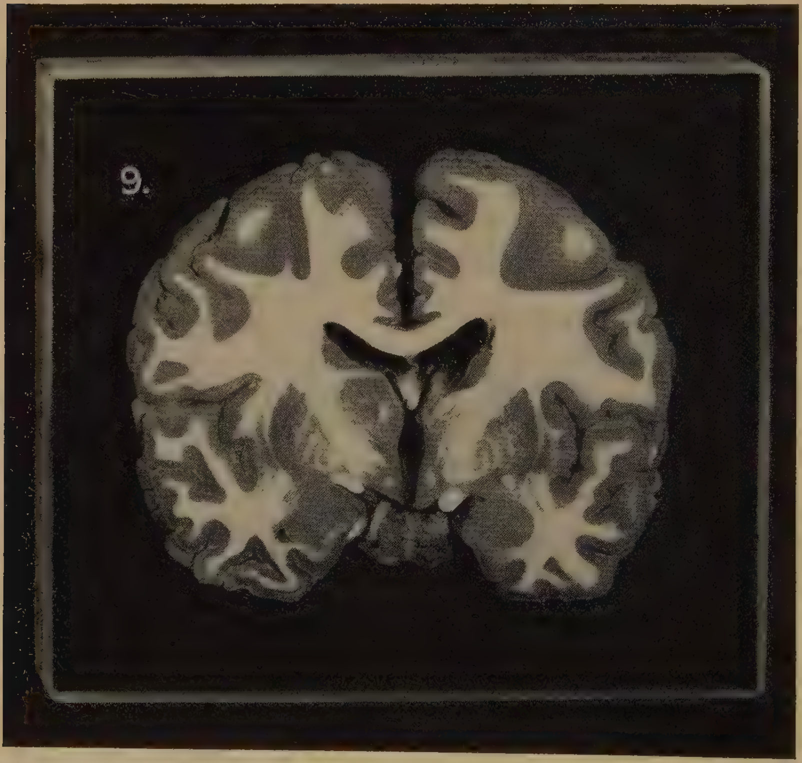


Fig. 1 (Ninth) coronal serial slice of a human cadaver brain, which had been emblamed with alcohol-glycerin-phenol, hardened in formalin and sectioned (8 mm thick) in reference to the Frankfurt plane, then dyed according to the modified Berlin-blue method, sugar-treated and surface-dried, and embedded as a wet specimen in Castolite on a black basal layer. Approx. one-half natural size (block, 17 × 14.5 × 2 cm).

the Anatomy Teaching Museum. The methods devised and adopted were to be simple, should produce preparations that are duplicated easily by other workers and make use of laboratory material which is routinely preserved and available.

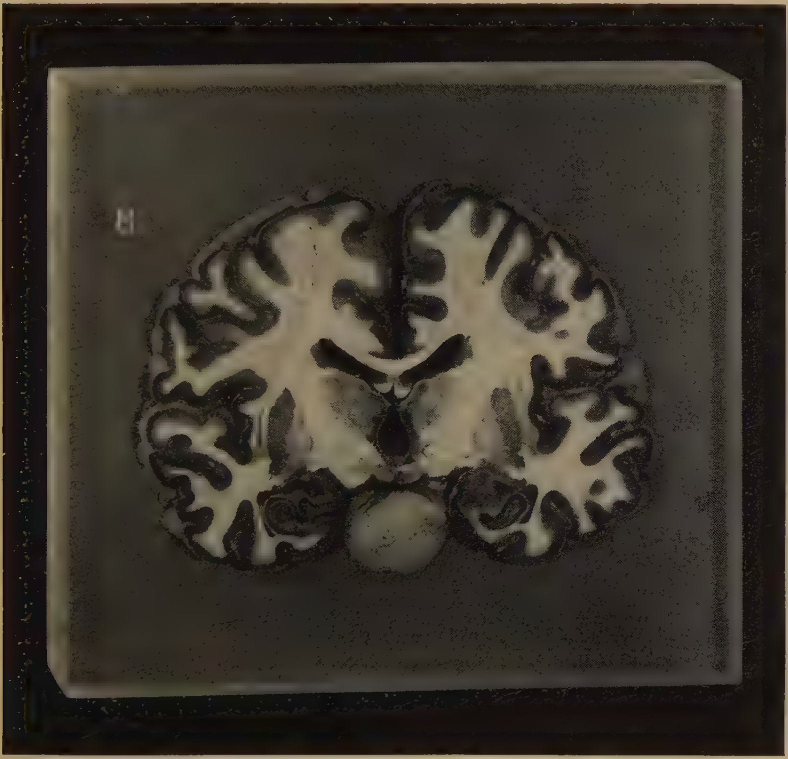


Fig. 2 (Eighth) coronal serial slice of a human cadaver brain, prepared as for figure 1, except for staining according to Brown's method and embedding on a green basal layer. Approx. one-half natural size (block, 17.5 × 15 × 2.5 cm).

Early in our experiments we found that dog brains flushed with saline and fixed with formalin by perfusion in situ gave, as was to be expected, the cleanest or best differentiations with various methods of gross staining and with subsequent mounting in plastic. But human brains properly fixed in situ by perfusion are rarely at the anatomist's disposal. Even the second best procedure, namely flushing and perfusing through the stumps of the carotid and vertebral arteries the brain removed at autopsy, is usually fraught with difficulties. For his teaching material the anatomist must depend on cadavers, which are generally embalmed initially with fluid containing glycerin, alcohol and phenol, after which the brain is removed for further fixation and hardening by immersion in formalin.

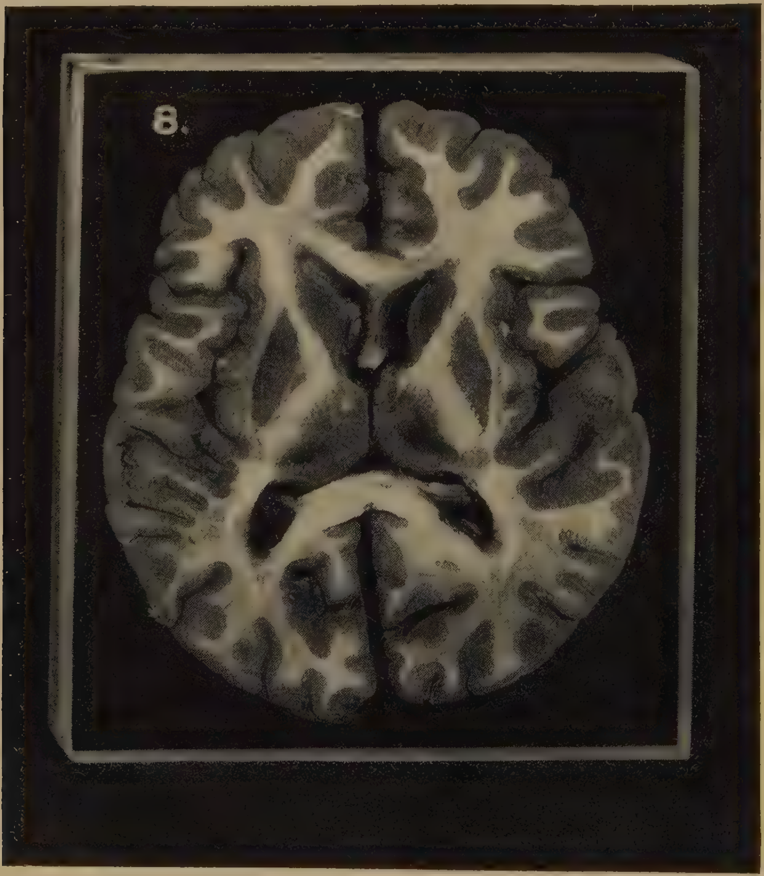


Fig. 3 Eight) horizontal serial slice of a human cadaver brain, prepared as for figure 1. Approx. one-half natural size (block, 16.5 × 14.5 × 2.5 cm). Note the blotchy appearance of the slice, especially of the white substance, because of the uneven diffusion of blood pigment (?) through it before or during embalming.

Obviously the initial embalming fluid, because of its composition, will affect the myelin material of the brain. The partial dissolution of this material will be reflected in the defective contrast staining between white and gray matter and lead to other imperfections which may not appear until after the specimens are mounted in plastic. For example, frequently a cadaver brain that appears excellently preserved externally will, upon slicing and staining, show a spotty or even a massive discoloration of the white matter (fig. 3) because of the diffusion of blood pigments through it. Such defects, accentuated in the plastic-embedded preparations, were pointed out in the differently stained brain sections exhibited (Kampmeier) at the annual meeting of the American Anatomists at New Orleans in April, 1950.

Considering first the former of the two defects, just indicated, some of our completed trial specimens of human cadaver brains showed a white haze or deposit here and there over the outside of the gyri. Chalky-white patches were seen over the cut surfaces of others. This debris was not visible grossly during the washing and staining of the slices and did not manifest itself until after the polymerization of the plastic in which the specimens were mounted. Possibly the explanation is that a fatty film, resulting from dissolved myelin, collects in diffuse patches or contracts into more circumscribed pools on the surface during air-drying of the specimen preliminary to such embedding.

We succeeded in getting rid of these superficial deposits as an incidental result during an experiment which aimed at quite a different problem. The aim was to find a way of avoiding the marked shrinkage of the brain — contraction of the gyri and wide opening of the sulci and fissures — that occurs as the surfaces of the specimen are subjected to prolonged drying while it is being prepared for mounting in plastic. Treatment of the specimen with a concentrated sugar solution prior to such mounting is apparently one of the means of overcoming some of the obstacles encountered.

The use of sugar in our method rests on an accidental observation, and no adequate explanation is offered here for its rationale. About a year ago the senior author noted that the contrast between the gray and the white substance of the brain is enhanced by soaking an undyed slice in corn syrup for a day or more, though the gain in contrast was lost upon embedding the preparation in plastic. But it was found: (1) that it was not necessary to air-dry the surfaces of a sugar-wet specimen as greatly as those of a water-wet specimen preparatory to plastic embedding, and (2) that the "fatty" deposits did not appear on such embedded specimens. It is likely that further experience and experiments will eventually and advantageously replace the syrup with other solutions of similar viscosity or comparable chemical qualities. It may be, too, that the procedure of sugar-curing can be adapted to the mounting of other biological wet preparations in plastics.

Against the second of the frequent imperfections in slices of cadaver brains mentioned above, namely the discoloration of the white substance, we are unable, at present, to offer a remedy, despite our experiments with bleaching fluids. Until a good bleaching method is developed, or unless a brain, which has been properly washed and fixed by perfusion, is obtainable, the museum preparator can only select the best cadaver brains, preserved in the usual way, and hope that one of them, after slicing, will yield a satisfactory series for gross staining and mounting. Hence, in this regard the recommendation of the trial-and-error method is essentially the same advice as given by other preparators (cf. Barnard, Roberts and Brown, '49).

The thickness of both the coronal and the horizontal serial slices of the human brain, prepared for our teaching collection and represented here, is approximately 8 mm each. It is obvious that this thickness is an arbitrary choice and that sections thinner or thicker can be stained grossly and mounted in plastics with equal facility. Uniform slices were cut in reference to the Frankfurt plane with a "macrotome" devised by Kampmeier and Bouldin ('50). The brain must be well hardened before slicing. Another precaution, also emphasized by previous workers, needs repeating; the knife, besides being sharp, must be drawn in a smooth, single, undeviating stroke through the brain to avoid rippling or leaving other marks of the blade. Such marks will interfere with even differentiation in staining.

Preparatory to gross staining, the slices are to be cleaned carefully by stripping away with tweezers the pia mater and its blood vessels on the surface of the gyri and in the depths of the sulci.

Staining

Many experiments were conducted in subjecting slices of the brain to different stains and to diverse chemical reactions. Among the stains tried were eosin, orange G, borax carmine, acetocarmine, Ziehl-Nielsen's carbolfuchsin, nigrosine, Harris' haematoxylin, and Weigert's iron haematoxylin. Though fair results were obtained with some of them, particularly the haematoxylins, none was able to produce the clear-cut differentiations between gray and white substance and the relatively permanent preparations as some of the physicochemical reactions with metal salts. Of the latter reactions Landau-Sincke's Berlin blue (iron chloride and potassium ferrocyanide) method and Brown's (lead nitrate, silver nitrate and ammonium sulphide) method gave the best preparations for plastic embedding.

In dyeing the brain slices with the Berlin blue reaction, we followed closely LeMasurier's ('35) modification of Landau-Sincke's technique. It is given here briefly, as follows: A slice is washed in running water for 12–14 hours and is then transferred for one hour to distilled water, changed three times. After this it is placed for two minutes in hot (60–50°C.) Mulligan's phenol solution ('31) in a quantity sufficient to cover the specimen to a depth of 6–7 cm. (The formula for Mulligan's solution is: 4% phenol crystals; 0.5% copper sulphate crystals, and 0.125% HCl dissolved in distilled water.) The slice is now immersed in a large volume of cold tap water for one minute, then in a 1% solution of ferric chloride in distilled water for two minutes, and washed in running water for 5 minutes. Following this it is treated with a 1% solution of potassium ferrocyanide in distilled water until the gray matter turns brilliantly blue, a differentiation that should not require more time than three minutes, after which it is washed in running water for 24 hours. Finally such slices are preserved in 5 or 10% neutral formalin; in this respect our procedure differs from that recommended by LeMasurier who kept his specimens in 70% alcohol. The slices remain in formalin indefinitely (at least 24 hours) until their transference to the sugar solution prior to their embedding in plastic.

Careful handling of the brain slices, a precaution pointed out also by previous observers, must be heeded during their staining. Since the phenol solution brings about a protective film of myelin over the white matter, thus keeping this substance from taking up the stain, any rough treatment or touching may abrade its surface and cause smudging of the tissue. Consequently we handle our slices with a broad spatula, lowering them into the dishes that contain the solutions rather than pouring the solutions over them.

When preparing brain slices for gross staining according to Brown's method, we treated them a little longer and at somewhat higher temperatures with carbolic acid solution than Brown recommended in his paper ('39). By doing so, a heavier protective film was produced over the white matter. Briefly, the course of the method is as follows: Upon removal of the slices from 10% formalin, they are washed for 12–24 hours in running water. They are then immersed in 5% phenol solution at 70–75°C. for 5–10 minutes, after which they are transferred to warm water (40–50°C.) for 5–10 minutes and to cold water for a few minutes for the purpose of hardening the phenol coating over the white matter. Next the slices are put for one minute into a solution of equal parts of 1% lead nitrate and 1% silver nitrate (a few drops of concentrated nitric acid to be added immediately after mixing). After being washed for 10–15 minutes in running water, they are dipped into 5–10% ammonium sulphide (we prefer the weaker solution) until good differentiation is obtained, which requires no more than 15–20 seconds. Finally, the slices are washed again in running water and placed in 10% formalin (instead of gelatin, as used by Brown) until their treatment with sugar, preparatory to their embedding in plastic.

Sugar curing

After our initial experiments of soaking the trial specimens in commercial corn syrup, we refined the method by using a saturated solution (approximately 85%) made by dissolving pure granulated sugar in warm distilled water.

Taking the brain slices from the formalin in which they were kept after staining, we blot their surfaces gently with absorbent towel paper to remove the excess formalin, and they are then immered in individual dishes containing the sugar solution. The slices remain in this hypertonic syrup for 3–6 days; if they stay in it too long, shrinkage becomes evident, as seen in the depression of the gray matter. Hence the slices should be observed from time to time and the sugar treatment terminated before that effect becomes too marked.

Mounting

Although the methods published three years ago by Kampmeier and Haviland remain much the same for the mounting of most of the different kinds of anatomical preparations in plastics, the modifications introduced here for the brain slices are sufficiently far-reaching to warrant a listing of all the steps in sequence, even at the expense of some repetition.

Before presenting such a recipe, the receptacles or molds into which the liquid plastic is to be poured for the embedding of the specimens deserve mention. We have discarded the use of glass dishes, metal pans and cardboard boxes because of various drawbacks (breakage, warping, unsuitable sizes, adherence of plastic, etc.), and resort now to wooden boxes properly constructed. Their side walls are made of planed strips, 2 cm ($\frac{3}{4}$ inch) thick, and the bottom or floor of plywood, 8 mm thick, all *screwed* together. (After the process of embedding the removal of the screws and consequent separation of the walls of the box frees the plastic block without cracking.) Since the brain slices embedded by us do not exceed 1 cm in thickness and the finished blocks are approximately 2.5 cm, the height of 4.5 cm ($1\frac{3}{4}$ inches) for the side-strips of a box is ample. The inside dimensions of the boxes used by us for the embedding of complete slices of the adult human brain are: depth, 4.5 cm; width, 15.5 cm; length, 18 cm. These dimensions accomodate both the coronal and the horizontal slices, permitting subsequent trimming and polishing of the block without undue waste of plastic, yet maintaining an esthetic proportion of border, if the slices are properly centered at the time of embedding.

To prevent the adhesion of the plastic to the wood, the inside of a box needs to be coated. A sufficient quantity of latex is either sprayed or slushed back and forth in the box to touch every part of its inner surface, and the excess is drained off. After the coating has dried, a film of ''Castoseal,''[1] which is to serve as a separating medium, is applied in the same way. Both the latex and this separator coat require each about one-half hour for drying.

To mount brain slices in plastic, we have altered our method, too, by using a ''cold-pouring'' Castolite, which contains about 7% styrene to make it more fluid and thus facilitate both the mixing of the catalyst with it and the displacement and removal of air bubbles emerging from the specimen during embedding. The earlier type of Castolite was heated in a paraffin oven to about 60°C. to make it more fluid for pouring. Such a Castolite would set, that is, polymerize, within one or two hours with considerable evolution of heat. The ''cold-pouring'' Castolite, on the other hand, generates relatively little heat during polymerization and is, therefore, less detrimental to the specimen. But the time for polymerization is prolonged considerably, not being complete until two or three days; preferably a longer time is allowed to elapse before trimming and polishing the block. At no time do we now subject a block containing a stained brain slice to "curing" in a paraffin oven, as recommended in the earlier procedure (1948).

---

[1] The plastic ''Castolite'' and the auxillary substances, such as the catalysts (''hardener'' and ''promotor''), the ''Castoseal'' and the color pigments, were purchased from Mr. William Weers, Castolite Company, Woodstock, Illinois.

Immediately before pouring the Castolite, two catalysts — the "hardener" and the "cold-setting promoter" — are added and thoroughly stirred into it. We add 15 drops of the "hardener" and 7–8 drops of the "promoter" to 100 cm^3 of the plastic, except for the anchoring layer (vide infra) which is reinforced by the addition of a little more of these substances.

Turning now specifically to the important steps in the production of a finished block containing the embedded brain slice, they are arranged in sequence, as follows:

1. Preparation of the base or background: Castolite, to which the catalysts have been added, is poured into the wooden boxes. For the dimensions of these receptacles given above, 300 cm^3 of the plastic will produce a layer about 1 cm thick. Such a basal layer or background may be transparent, or may, as we prefer, be opaque, in which case an appropriate pigment in sufficient quantity is stirred into the resin before pouring. Thus, black represents an attractive base for the brain slices dyed with Berlin-blue, and green for those dyed according to Brown's method. Not only do such color contrasts enhance the appearance of the specimens (figs. 1–3), but in the case of serial slices, where the anterior or upper surface of one is the same as the posterior or lower surface of the slice next in front or above, the opaque bases covering, say, the posterior or the lower surfaces will guard against the student's confusion which could result if a transparent base were used and the bilaterally symmetrical sections were reversed during his handling of them.

2. Surface drying of the sugar-treated brain slice: A slice taken from the syrup is rinsed for a few seconds in a trickle

of water from the faucet to remove the excess sugar on its surface and thus make it less sticky during drying. After the slice is blotted gently with a paper towel, it is air-dried with an electric fan for 10–15 minutes, stopping before it tends to turn brown from drying. Obviously both sides must be treated alternately in this way. To avoid abrasions of the superficial film of color during such manipulations it is well to lay the slice on a sheet of paper towel, then to apply a sheet on the exposed surface before turning it over, followed by the removal of the sheet from the upturned side that is to be subjected to the air current.

3. Centering and anchoring the brain slice on the base: A slice — with the posterior face (if coronal section), or inferior face (if horizontal section), down — is laid on the basal plastic layer and is properly oriented with respect to the borders of the box. (It is obvious that two or three slices, taken at the poles of the brain, can be mounted in sequence on the same base, if the bases are uniform in size.) A small volume, about 50 cm^3, of the fluid plastic, to which proportionately more of the ''hardener'' (10 drops) and ''promotor'' (6 drops) have been added, is poured around — *not over* — the slice. The increased quantity of the catalysts speeding the polymerization of the resin, the brain slice becomes firmly adherent to the base within half an hour. During this setting the box should be covered to protect the exposed surface of the slice from either drying or absorption of moisture.

4. Pouring the covering layer: The preceding step, if done correctly, will have fastened the specimen so that it will not rise when the embedding or covering layer is poured over it. Not more than 150 cm^3 of the plastic is necessary for this stratum; it has been prepared, of course, by stirring into it the catalysts (''hardener,'' 15 drops per 100 cm^3, and ''promotor,'' 7–8 drops). The slower setting of the plastic will allow the air, caught in the crevices of the brain, to float to the top as bubbles which can easily be punctured or brushed aside. Any bubbles visibly trapped in the depth are readily displaced also with a probe. Small bubbles that may form

between the lower surface of the slice and the plastic base can be disregarded if that background is opaque. After 24 hours, the polymerization of the stratum of plastic in which the brain slice is embedded is virtually complete and permits the next stage in the making of the block.

5. Pouring of the final or surface layer: Approximately 250–300 cm^3 of the prepared plastic will furnish a top layer of adequate depth. If the quantities indicated in the preceding steps of the procedure have been employed, the three strata — the basal, the anchoring and embedding, and the final or viewing — will then have about equal thickness and will counteract any remote tendency to bowing or warping of the block. At the end of the day when the third or top stratum has set, a sheet of thin cellophane is applied to the sticky surface of the plastic and gently pressed down and smoothed with the fingers without imprisoning air bubbles under it. The adhering cellophane, by excluding the air, accelerates the hardening of the superficial film of plastic, which otherwise would remain sticky for a long time.

6. Removal of the block from its mold: After two to three days, or preferably longer, when the reactions of polymerization and curing can be regarded as complete, the block is ready for the final steps of processing. To extricate the block, the removal of the screws from one or two of the sides of the box will commonly suffice; less often the entire box needs to be unscrewed to separate the adhesion of the plastic to the wood. Stripping off the cellophane from the top surface frees the block entirely from its encasement.

7. Trimming and finishing the block: The methods of squaring, trimming and polishing the plastic block, so that it will display the embedded anatomical preparation to the best advantage, have been detailed by the senior author in the earlier paper ('48) and need not be described here. Needless to say, too, that if the appropriate machines — bandsaw, lathe, sander, buffing wheels, etc. — are available, these final proc-

esses will be accomplished more speedily although a perfect block can be achieved as well with the simplest of hand equipment.

SUMMARY

The procedure of preparing serial slices of human cadaver brains as wet specimens, mounted in the transparent synthetic resin Castolite (of "cold-pouring type"), is described under 4 headings:

Slicing. Selected and formalin-hardened brains are cut, in reference to the Frankfurt plane, with a macrotome into slices (either coronal or horizontal) of uniform and measured thickness.

Staining. Of the different methods of gross staining of the brain slices tried, the modified Landau-Sincke's Berlin blue reaction and Brown's reaction yielded the best preparations.

Sugar curing. Prior to embedding, the stained slices are treated with a concentrated sugar solution; this treatment prevents shrinkage during the subsequent air-drying of the specimen.

Mounting. The successive steps in the production of the plastic block, with the brain slice mounted in it, are described; they are as follows: construction of the embedding boxes, preparation of the basal plastic layer, surface drying of the brain slice, centering and anchoring of the slice on the base, pouring the covering layer, pouring the surface layer, removal of the block from the mold, and its trimming and finishing.

LITERATURE CITED

Barnard, John W., J. O. Roberts and Joseph G. Brown 1949 A simple macroscopic staining and mounting procedure for wet sections from cadaver brains. Anat. Rec., *105:* 11–18.

Blair, D. M., F. Davies and E. W. McClelland 1932 On the nature of certain macroscopic staining reactions of the brain. J. Anat., *66:* 478–485.

Brown, Donald B. 1939 Macroscopic staining of the brain. Anat. Rec., *75:* 419–425.

Green, H. L. H. H. 1933 The preparation of serial macroscopic brain sections for teaching purposes. J. Anat., *67:* 346–349.

KAMPMEIER, OTTO F. 1933 Some simple methods of preparing and mounting gross sections of the body, of illustrating microscopic sections, and of labelling specimens for the anatomical museum. Anat. Rec., *65:* 395–401.

——— 1950 Stained gross sections of the brain, cut with reference to the Frankfurt plane (demonstration). Am. Assoc. of Anat., 63rd Session, April 5–7, New Orleans, Anat. Rec., *108.*

KAMPMEIER, OTTO F., AND RALPH F. BOULDIN 1950 Sectioning the human brain with reference to the Frankfurt plane. Anat. Rec., *107:* 415–422.

KAMPMEIER, OTTO F., AND THOMAS N. HAVILAND 1947 Anatomical specimens mounted in plastics (demonstration). Am. Assoc. of Anat., 60th Session, April 3–5, Montreal, Canada. Anat. Rec., 98. Demonstrations of larger exhibits at 107th annual meeting of Illinois State Med. Soc., Chicago, May 12–14; at 96th annual session of Am. Med. Assoc., Atlantic City, June 9–13, and at the Internat. Sci. Exhib. of the 114th annual meeting of A.A.A.S., December 26–31.

——— 1948 On the mounting of anatomical museum specimens in transparent plastics. Anat. Rec., *100:* 201–232.

KAMPMEIER, OTTO F., THOMAS HAVILAND, MAX M. STRUMIA AND J. IVAN HERSHEY 1950 Plastics: Mounting of Biologic Specimens. Medical Physics, Vol. II (Otto Glasser, editor; Year Book Publishers, Inc., Chicago), pp. 719–728.

LANDAU, E. 1919 Über chemische Differenzierungen an makroskopischen Gehirnschnitten. Schweiz. Arch. f. Neur. u. Psychiat., *5:* 68–69.

LEMASURIER, H. E. 1935 Simple method of staining macroscopic brain sections. Arch. of Neur. and Psychiat., *34:* 1065–1067.

LENNHOFF, CARL 1910 Beitrag zur Histotechnik des Centralnervensystems. Neurol. Centralblatt, *29:* 20–22.

MAINLAND, O. 1928 Über makroskopische Färbung von Gehirnpräparaten mit Berlinerblau. Anat. Anz., *65:* 84–88.

MULLIGAN, J. H. 1931 A method of staining the brain for macroscopic study. J. Anat., *65:* 468–472.

SINCKE, GUSTAV 1926 Eine neue Methode zur Färbung von makroskopischen Gehirnschnitten. Anat. Anz., *61:* 311–313.

SPATZ, H. 1922 Über Stoffwechseleigentümlichkeiten in den Stammganglien. Zeitschr. f. d. ges. Neur. u. Psychiat., *78:* 641–648.

ENDOCRINE CONTROL OF LYMPHOID TISSUE[1]

J. D. FELDMAN[2]

Public Health Service Postdoctorate Research Fellow in the Department of Pathology, Yale University School of Medicine, New Haven, Connecticut

EIGHTEEN FIGURES

INTRODUCTION

The object of this report is to present experimental data on the effects of growth hormone upon lymphoid tissue and the role of the pituitary gland in regulating lymphoid tissue mass and structure.

The concept of adrenal cortical control of lymphoid tissue has been elaborated over the past decade by numerous investigators (Dougherty and White, '44, '45; Selye, '46; Valentine et al., '48). It has been repeatedly demonstrated that increased activity of the adrenals, produced either by stress or by administration of ACTH, or injection of purified cortical compounds results in a shrinkage of lymphoid tissue following destruction of lymphoid elements (Dougherty and White, '44, '45; Evans et al., '38; Ingle et al., '38, '38a, b; Selye, '46). Diminution of adrenal activity, effected by adrenalectomy, results in a hyperplasia of lymphoid organs (Houssay, Del Castillo and Pinto, '41; Jaffe, '24, '27; Reinhardt and Holmes, '40; Simpson, Dennison and Korenchevsky, '34).

Similarly, the gonads affect size and weight of lymphoid tissue. There are several investigations which have shown that increased activity of the gonads, produced by gonadotrophins, or the administration of purified sex hormones

---

[1] This work was supported by a research grant from the National Institutes of Health, U.S.P.H.S.

[2] Present Adress: Institute of Pathological Anatomy and Histology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

causes a diminution of lymphoid organs (Evans and Simpson, '34; Korenchevsky, Dennison and Simpson, '35; Reinhardt and Wainman, '42). In the opposite direction castration results in hyperplasia (Lauson, Golden and Sevringhaus, '39; Marine, Manley and Baumann, '24; Chiodi, '40; Reinhardt and Wainman, '42).

The alterations in the spleen, thymus and lymph nodes which occur after the administration of growth hormone have not been described, nor has the histology of lymphoid hyperplasia following adrenalectomy been reported in detail, except for the thymus (Jaffe, '24). The data to be presented indicate that the hyperplasia after adrenlaectomy is primarily due to the unopposed action of growth hormone and not to the absence of lympholytic cortical steroids. The data also show that the thyroid has no direct influence upon these organs.

MATERIAL AND METHODS

Male Sprague-Dawley rats, 14–15 weeks of age, were used in all experiments. The animals were fasted for 72 hours before sacrifice to obviate differences in food intake. Water was allowed ad libitum. Experimental and control groups were handled simultaneously and autopsied at the same time.

The experiments were directed toward three lines of investigation.

I. To study the effects of growth hormone, 14 hypophysectomized rats, of the same age, received from a commercial laboratory[3] and operated on at 70 days of age, were divided into two groups of 7 each. One group of animals was given a total of 9 mg of the hormone and sacrificed 24 hours after the last dose (28 days after hypophysectomy). Purified growth hormone[4] was prepared as a 0.5% solution in isotonic saline. It was injected intraperitoneally in 0.2 ml doses three times daily for three days. The second group of hypophysectomized rats, serving as controls, was given isotonic saline intraperitoneally in the same doses and over the same period.

---

[3] Hormone Assay Laboratories, Chicago, Illinois.

[4] Purified growth hormone was kindly supplied by Dr. Alfred Wilhelmi, Department of Physiological Chemistry, Yale University School of Medicine.

A third group of intact rats, of the same age, was injected with isotonic saline intraperitoneally in the same doses and over the same period of time. They served as controls for the hypophysectomized animals.

II. To study the effects of adrenalectomy, 7 normal rats were adrenalectomized at the age of 96 days and were sacrificed 7 days later. Thirteen intact rats served as controls. Both groups received 1% saline for drinking fluid.

III. To study the effects of the thyroid gland three experimental groups of rats were employed. One group of 6 intact animals, 98 days of age, was given subcutaneously 300 μg of crystalline thyroxin[5] daily for 7 days. The thyroxin was prepared in 0.001 N NaOH and injected once daily. A control group of 6 animals was injected with 0.001 N NaOH alone.

Six normal rats were thyroidectomized at 71 days of age and were sacrificed 30 days later. They were given 1.8% calcium lactate and 0.2% calcium chloride for drinking fluid. A control group of 6 rats received the same drinking water.

Three rats, hypophysectomized at 70 days of age and sacrificed 34 days later, were injected with crystalline thyroxin as above. A control group of three hypophysectomized animals were given 0.001 N NaOH subcutaneously.

All animals were etherized and exsanguinated. The spleen and thymus were immediately removed, cleaned of fat and each promptly weighed on a torsion balance. The organs were sliced in 2–3 mm wide blocks and placed in appropriate fixatives. The 6 largest mesenteric lymph nodes were cleaned of adventitial tissue, weighed together, and put into fixing solutions. The entire procedure required 12 minutes or less.

A single slice of spleen and thymus and one or more lymph nodes were fixed in each of the following solutions: (1) 10% buffered formaldehyde, pH 7.2, for fat stains (Lison, '36) and for detection of iron (Bunting, '49); (2) equal parts of 6% aqueous mercuric chloride and 95% alcohol, for phloxine methylene blue and Feulgen nucleal stains (Lison, '36); (3) 9 parts of saturated picric acid in absolute alcohol and 1 part of 40% formaldehyde for glycogen by the Bauer-Feulgen method (Lison, '36).

---

[5] Roche-Organon, New Jersey.

In each splenic section 10 follicles and perifollicular envelopes (Krumbhaar, '49) selected at random, were measured in the longest and shortest diameters with a calibrated micrometer eyepiece. Follicle size was obtained by averaging all the measurements of a group of animals. The perifollicular envelope was measured at 4 points around each follicle and all the measurements of one group of spleens averaged.

OBSERVATIONS

Spleen

The spleens of intact fasted control rats consisted of the following structures:

1. The follicle was composed predominantly of small lymphocytes (fig. 1). It contained a few large cells with phagocytic properties, usually eccentrically located in small clusters or as scattered single cells. The average follicle size was 470 × 304 μ on cross section (table 2). The periphery of the follicle was encircled by a thin connective tissue capsule in which one or more capillaries were present. Along the inner border of the capsule was a disconnected row of large basophilic cells, some of which were phagocytic for hemosiderin.

2. The perifollicular envelope (Krumbhaar, '49), varying from 8 to 12 cells in width and averaging 46 μ, encircled the follicle and was separated from it by the capsule (fig. 1). The cells of the envelope, both in fixed tissue sections and in air-dried prints, were phagocytic for India ink particles. Within the zone occasional polymorphonuclear neutrophils and red blood cells were seen, but macrophages and hemosiderin were absent.

3. The pulp constituted the remainder of the organ. It was composed of sinuses, reticulum, trabeculae, blood vessels and cells. The cellular elements were heterogeneous and

included lymphoid cells, numerous macrophages, scattered megakaryocytes, red blood cells, occasional polymorphonuclear neutrophils and plasma cells, myeloid and reticulum cells.

Iron stains revealed the presence of a moderate amount of hemosiderin, mostly within macrophages and predominantly in the pulp. A few particles of hemosiderin were occasionally found in phagocytic cells within follicles and along the inner border of the follicular capsule. None was seen in the perifollicular envelope (fig. 7).

Evidence of cellular breakdown, manifested by pyknosis and nuclear particles in Feulgen preparations, was slight. In a few phagocytes within a few follicles one or more Feulgen positive fragments were visible.

Sudan IV and sudan black B stains of the spleen in all groups were consistently negative and glycogen was found only in the cytoplasm of polymorphonuclear neutrophils.

Following hypophysectomy the spleen shrank to approximately half the size and weight of the intact controls (table 1). Histologically all structures appeared atrophied. The follicles, averaging 372 × 247 μ, were small, compact and appeared close together. The follicular cells were small, darkly stained and showed little nuclear detail. The perifollicular envelope was diminished in width, averaging 20 μ. Its cells were smaller and more deeply stained than those in normal controls (fig. 2). The number of megakaryocytes was markedly reduced and the amount of hemosiderin was increased (fig. 8). The degree of tissue breakdown, according to the criteria mentioned above, was approximately the same as in intact fasted controls.

The administration of growth hormone to hypophysectomized rats resulted in an overgrowth of lymphoid elements and an enlargement of the spleen (table 1). The most striking change was in the perifollicular envelope and pulp. These two regions blended together due to the presence of almost solid sheets of large lymphoid elements (fig. 3). The cells had large pale nuclei and abundant cytoplasm varying from basophilia to acidophilia (fig. 6). Mitoses were numerous.

TABLE 1

All numbers, except those in the first column, are averages for the group. Significant differences between groups are noted by italicized numbers. P values of .05 or less are regarded as significant

	NO. OF ANIMALS	BODY WT. AT AUTOPSY	AGE IN DAYS	SPLEEN	SPLEEN B.W.	THYMUS	THYMUS B.W.	LYMPH NODES	LYMPH NODES B.W.
					mg%		*mg%*		*mg%*
Intact controls	6	256	104	*_644_[1]	251	_264_	_103_	299	*_117_[1]
Hypophysectomized	7	173	98	+_384_[2]	_222_	_190_	_145_	339	+_194_[2]
Hypophysectomized plus growth hormone	7	155	98	+_485_[3]	_311_	175	113	338	+_219_[3]
Adrenalectomized	7	310	103	_865_	_279_	_423_	_134_	+_387_	125
Intact controls	13	267	103	_616_	_231_	_269_	_101_	+_289_	108
Thyroxin administr.	6	217	98	729	_334_	_208_	96	232	105
Controls	6	256	98	601	_235_	_369_	144	231	90
Thyroidectomized	6	257	101	570	222	264	102	319	124
Controls	6	263	101	598	223	276	105	311	118
Hypophysectomized plus thyroxin	3	127	104	340	266	147	116	248	193
Hypophysectomized controls	3	160	104	329	210	186	116	236	148

* Determination of significance was calculated between 1 and 2, and 2 and 3.

+ P equals .05.

Macrophages, hemosiderin and red blood cells in the pulp were practically absent, having been replaced by the new lymphoid cells (fig. 9). Megakaryocytes were difficult to find. In the follicle all cells were larger than in hypophysectomized controls and the follicular phagocytic cells were increasd in number. Follicles averaged 447 × 279 μ. Evidence of cellular breakdown as revealed by the presence of intracellular nuclear debris appeared to be of the same order as in intact and hypophysectomized controls.

TABLE 2

Data on size of splenic follicle and mantle zone in experimental and control animals

GROUP	NUMBER OF OBSERVATIONS	μ FOLLICLE SIZE	MANTLE ZONE WIDTH μ
Unoperated controls	60	470 × 304	46
Hypophysectomized	70	372 × 247	20
Hypophysectomized + growth hormone	70	447 × 279	not measurable
Adrenalectomized	70	487 × 321	87
Controls	130	459 × 303	71
Unoperated + thyroxin	60	438 × 276	54
Controls	60	444 × 315	47
Thyroidectomized	60	484 × 307	56
Controls	60	474 × 308	47
Hypophysectomized + thyroxin	30	412 × 280	24
Hypophysectomized controls	30	389 × 283	33

The histological appearance of the spleens of adrenalectomized animals was similar to that of hypophysectomized animals treated with growth hormone, but the proliferation was not so marked. Table 1 presents the data of this group and their controls. The spleen had increased significantly in weight and size over that in intact controls. Histologically the perifollicular envelope was greatly widened, though not to the extent observed in the spleens of animals injected with growth hormone, and its cells had the same cytological characteristics as were seen in the spleens of growth hormone treated rats (fig. 4). The perifollicular envelope averaged 87 μ in width. Mitoses were numerous. The pulp contained more cellular elements, especially of the lymphoid type, than in control spleens, and sinuses were filled with erythrocytes. Megakaryocytes were reduced in number but not to the extent seen in spleens of hypophysectomized animals. The amount of hemosiderin was approximately the same as was found in intact controls (fig. 10). Follicles were large and averaged 487 × 321 μ. They were composed of lymphocytes which were increased in size and which had abundant cytoplasm.

The presence of excess thyroxin or the absence of the thyroid in both intact and hypophysectomized rats resulted in no demonstrable histological alterations of splenic structure. It is worth noting, however, that the thymus of thyroxin injected rats was enlarged and that on the basis of body weight the spleen was also significantly heavier than in controls. In hypophysectomized animals, the administration of thyroxin did not result in any weight changes different from hypophysectomized controls.

Thymus

The thymus of intact fasted controls consisted of a cortex composed chiefly of compact small lymphocytes, scattered reticulum cells, septa and contained vessels. The medulla, sharply separated from the cortex, was of looser texture and was composed of lymphocytes, reticulum cells, Hassall's corpuscles, scattered polymorphonuclear neutrophils and mast cells, and a loose vascularized reticulum (fig. 11). A few pyknotic cells were visible in every low power field and ingested nuclear particles were also found in some phagocytes, both in the cortex and medulla. No visible fat or iron was seen in the thymus from all groups, and glycogen was present only in the cytoplasm of polymorphonuclear neutrophils.

Following hypophysectomy the thymus atrophied and weighed significantly less than that of intact controls (table 1). Histologically the cortex and medulla were shrunken and all the cellular elements appeared small, deeply stained, with loss of nuclear detail (fig. 12). Other than these changes the thymus was much the same as found in intact controls.

With administration of growth hormone there was no increase of thymic weight over hypophysectomized controls but microscopically the cortex and medulla appeared widened and the cells, particularly lymphoid, were large and apparently newly formed. The nuclei were pale and large and cytoplasm was abundant. Mitotic figures were frequently seen. In addition there was an increase in the number of cells in the medulla, and these had characteristics of large young lymphocytes (fig. 13). No other changes were visible.

The thymus of adrenalectomized rats was larger and heavier than the average of the control group. Microscopically the picture was indistinguishable from that seen in the hypophysectomized rat administered growth hormone (fig. 14).

In intact rats injected with thyroxin the thymus was significantly decreased in weight but microscopically the same as in controls. Thyroidectomy of intact rats and injection of thyroxin into hypophysectomized rats resulted in no demonstrable changes.

Lymph nodes

The lymph nodes of intact fasted controls consisted of the following structures:

1. Follicles were composed of an eccentric core of large basophilic cells, with the cytological features of large young lymphocytes and stem cells, surrounded by compact small lymphocytes.
2. Medullary cords were filled with large lymphoid elements, phagocytes, scattered plasma and reticulum cells, all embedded in a loose reticulum (fig. 15).
3. Peripheral and medullary sinuses contained relatively few cells of any kind but those present were mostly macro-

phages and some lymphocytes and polymorphonuclear neutrophils.

No fat or iron was found in any of the lymph nodes of any group, and as elsewhere, the glycogen was present only in the cytoplasm of polymorphonuclear neutrophils.

Cellular breakdown was represented by the presence of nuclear debris in phagocytes within follicles and sinuses. Pyknosis and cell fragmentation were generally more obvious in lymph nodes than in spleen or thymus.

Following hypophysectomy there was an increase of lymph node weight, which was significant only on the basis of body weight. Histologically the lymph nodes of hypophysectomized animals were not distinguishable from those of intact controls (fig. 16).

With administration of growth hormone, follicles and their germinal centers were increased in size and the cells were enlarged. Sinuses and medullary cords were filled with solid sheets of new large lymphocytes (fig. 17). These changes were not found throughout the nodes, but were spotty and localized. Mitoses were numerous, particularly in areas of large new cells. Cellular breakdown appeared to be the same as in intact and hypophysectomized controls. On the basis of body weight the lymph nodes of growth hormone treated rats were significantly heavier than those of hypophysectomized controls.

Adrenalectomy produced a histological appearance similar to that found in the lymph nodes of growth hormone treated animals and an increase in weight over the controls (fig. 18).

Thyroxin and thyroidectomy had little effect upon gross weight and microscopic picture.

DISCUSSION

Administration of growth hormone to hypophysectomized rats resulted in active proliferation of lymphoid tissue and hypertrophy. This effect might have been mediated through any one or more of the following pathways. (1) Depression of adrenal activity or withdrawal of effective concentration of adrenal steroids; (2) depression of gonadal function or withdrawal of effective concentration of gonadal hormones; (3) relative excess of insulin; (4) direct action of growth hormone on cellular metabolism.

The increase of lymphoid tissue in hypophysectomized animals treated with growth hormone was not due to a depression of adrenal cortical activity or of cortical steroids nor to a depression of gonadal activity or gonadal hormones as a result of removal of the pituitary. If this had been so, evidence of hyperplasia and hypertrophy should have been found in hypophysectomized rats without growth hormone, whereas these animals had atrophied lymphoid tissue.

It has been suggested that growth hormone causes nitrogen retention and protein synthesis by stimulating insulin production or that growth hormone requires a minimal effective concentration of insulin to ensure its anabolic action (Frame and Russell, '46; Gaebler and Galbraith, '41; Gaebler and Robinson, '42; Hoberman, '50; Mirsky, '39; Young, '45). At present it is not known whether growth hormone exerts its proliferative action via insulin or acts directly upon cellular metabolism. Latta and Henderson ('37) reported hyperplasia of lymphoid tissue following prolonged administration of insulin. The experiments of Evans, Simpson and Li ('48) suggest that the growth hormone effect is a direct one upon peripheral tissue, but the role of insulin was not determined.

In the present experiments the thyroid had no apparent direct influence on lymphoid tissue. Other investigators (Grollman, '38; Marine et al., '24; Marder, '49) have reported an increase of lymphoid mass following the administration of thyroxin, an involution of lymphoid tissue following thyroidectomy, or after both thyroidectomy and adrenalectomy. The data of this paper showed that when the pituitary was removed the injection of thyroxin resulted in no demonstrable differences from control hypophysectomized animals. Thyroidectomy of intact rats also was not associated with any apparent alterations of lymphoid tissue. Injection of thyroxin into intact animals yielded inexplicable results. The spleen and thymus increased in weight but the lymph nodes decreased. Reinhardt and Wainman ('42), on the contrary, reported an increase of lymph node and spleen weight after administration of desiccated thyroid to intact rats, and a decrease of thymic mass. The explanation for these different responses of lymphoid organs is not apparent. However, the conclusion seems necessary that whatever the changes in lymphoid tissue, they were mediated by the hypophysis.

It was of interest that the amount of hemosiderin present in the spleen increased following hypophysectomy and decreased after the administration of growth hormone. This was consistent with the reports that hypophysectomy caused an anemia in rats and that several pituitary factors, of which growth hormone was one, correctd the anemia (Crafts, '46; Gordon and Charipper, '47). The data, together with the findings of Crafts ('46) and Gordon and Charipper ('47), implied that after removal of the pituitary there was increased erythrocyte destruction with deposition of hemosiderin in the spleen. Growth hormone prevented this and consequently little or no hemosiderin was deposited in the spleen.

Finally the observation was made that megakaryocytes were reduced in number in the spleens of hypophysectomized rats and that this change was not corrected by injection of growth hormone. The explanation for this is unknown.

CONCLUSIONS

Administration of growth hormone to hypophysectomized male rats resulted in marked proliferation and hypertrophy of lymphoid cells and an increase in weight of the spleen and thymus. Hypophysectomy alone was followed by atrophy of both lymphoid organs and cells. Adrenalectomy resulted in hyperplasia and hypertrophy of lymphoid tissue histologically similar to that seen in growth hormone treated animals. The thyroid had only a slight influence upon lymphoid tissue and this appeared to be mediated through the pituitary.

Lymphoid tissue mass can be reduced and lymphoid cells can be destroyed by means of ACTH which controls adrenal activity and by means of gonadotropins which control gonadal activity. Lymphoid tissue mass can be increased and lymphoid cells show proliferation and hypertrophy after administration of growth hormone.

I am indebted to Dr. Henry Bunting, Department of Pathology, Yale University School of Medicine, for aid in preparing this work and to Miss Patricia Reynolds for help in preparing the tissues and slides.

REFERENCES

BUNTING, H. 1949 The histochemical detection of iron in tissues. Stain Tech., *24:* 109–115.

CHIODI, H. 1940 The relationship between the thymus and the sexual organs. Endocrinology, *26:* 107–116.

CRAFTS, R. C. 1946 Effects of hypophysectomy, castration, and testosterone propionate on hemopoiesis in the adult male rat. Endocrinology, *39:* 401–413.

DOUGHERTY, T. F., AND A. WHITE 1944 Influence of hormones on lymphoid tissue structure and function. The role of pituitary adrenotrophic hormone in the regulation of the lymphocytes and other cellular elements of the blood. Endocrinology, *35:* 1–14.

——— 1945 Functional alterations in lymphoid tissue induced by adrenal cortical secretion. Am. J. Anat., *60:* 423–435.

EVANS, H. M., M. E. SIMPSON AND W. R. LYONS 1938 Atrophy of thymus of the rat resulting from administration of adrenocorticotrophic hormone. Proc. Soc. Exp. Biol and Med., *38:* 419–420.

EVANS, H. M., M. E. SIMPSON AND C. H. LI 1948 The gigantism produced in normal rats by injection of the pituitary growth hormone. I. Body growth and organ changes. Growth, *12:* 15–32.

FRAME, E. G., AND J. A. RUSSELL 1946 The effects of insulin and anterior pituitary extract on the blood amino nitrogen in eviscerated rats. Endocrinology, *39:* 420–429.

GAEBLER, O. H., AND A. R. ROBINSON 1942 Effects of the pancreas and the adrenals upon production of nitrogen storage with pituitary preparations. Endocrinology, *30:* 627–634.

GAEBLER, O. H., AND H. W. GALBRAITH 1941 Effects of anterior pituitary preparations in experimental pancreatic diabetes. Endocrinology, *28:* 171–178.

GORDON, A. S., AND H. A. CHARIPPER 1946 The endocrine system and hemopoiesis. Bull. N. Y. Acad. Sci., *48:* 615–640.

GROLLMAN, A. 1936 The Adrenals. Pp. 176 et seq. The Williams and Wilkins Co., Baltimore.

HOBERMAN, H. D. 1950 Endocrine regulation of amino acid and protein metabolism during fasting. Yale J. Biol. and Med., *22:* 341–367.

HOUSSAY, B. A., E. B. DEL CASTILLO AND A. PINTO 1941 Accion de la suprarenalectomiz sobre el timo y los gånglios. Rev. Soc. Arg. de Biol, *17:* 26–39.

INGLE, D. J. 1938 Atrophy of the thymus in normal and hypophysectomized rats following administration of cortin. Proc. Soc. Exp. Biol. and Med., *38:* 443–444.

INGLE, D. J., G. M. HIGGINS AND E. C. KENDALL 1938a Atrophy of the adrenal cortex in the rat produced by administration of large amounts of cortin. Anat. Rec., *71:* 363–372.

INGLE, D. J., AND H. L. MASON 1938b Subcutaneous administration of cortin compounds in solid form to the rat. Proc. Soc. Exp. Biol. and Med., *39:* 154–156.

JAFFE, H. L. 1924 The influence of the suprarenal gland on the thymus. I. Regeneration of the thymus following double suprarenalectomy in the rat. J. Exp. Med., *40:* 325–341.

———— 1927 The suprarenal gland. Arch. Path., *3:* 414–453.

KORENCHEVSKY, V., M. DENNISON AND S. L. SIMPSON 1935 The prolonged treatment of male and female rats with androsterone and its derivatives, alone or together with oestrone. Biochem J., *29:* 2534–2552.

KRUMBHAAR, E. B. 1948 A hematopoietic perifollicular envelope in the rat spleen. Blood, *3:* 953–959.

LATTA, J. S., AND J. W. HENDERSON 1937 The hemopoietic disturbances induced in the albino rat by insulin administration. Folia Hematol., *57:* 206–227.

LAUSON, H. D., J. D. GOLDEN AND E. L. SEVRINGHAUS 1939 The rate of increase in hypophyseal gonadotropic content following ovariectomy in the rat, with observations on gland weights. Endocrinology, *25:* 47–51.

LISON, L. 1936 Histochimie Animale, pp. 189–220 and 176–181. Gauthier-Villars, Paris.

MARDER, S. N. 1949 Effect of thyroxine on lymphoid tissue mass of adult male mice. Proc. Soc. Exp. Biol. and Med., *72:* 42–45.

MARINE, D., O. T. MANLEY AND E. J. BAUMANN 1924 The influence of thyroidectomy, gonadectomy, suprarenalectomy and splenectomy on the thymus gland of rabbits. J. Exp. Med., *40:* 429–444.

MIRSKY, I. A. 1939 The influence of the anterior pituitary gland on protein metabolism. Endocrinology, *25:* 52–56.

REINHARDT, W. O., AND R. O. HOLMES 1940 Thymus and lymph nodes following adrenalectomy and maintenance with NaCl in the rat. Proc. Soc. Exp. Biol. and Med., *45:* 267–270.

REINHARDT, W. O., AND P. WAINMAN 1942 Effect of thyroidectomy, castration and replacement therapy on thymus, lymph nodes, spleen in male rats. Proc. Soc. Exp. Biol. and Med., *49:* 257–260.

SELYE, H. 1946 The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocrinol., *6:* 117–231.

SELYE, H., C. M. HARLOW AND J. B. COLLIP 1936 Über die Auslösung der Alarmreaktion mit Follikelhormon. Endokrinologie, *18:* 81.

SIMPSON, S. L., M. DENNISON AND V. KORENCHEVSKY 1934 Some effects of adrenalectomy in male rats. J. Path. and Bact., *39:* 569–590.

VALENTINE, W. M., C. G. CRADDOCK, JR. AND J. S. LAWRENCE 1948 Relation of adrenal cortical hormone to lymphoid tissue and lymphocytes. Blood, *3:* 729–754.

YOUNG, F. G. 1945 Growth and diabetes in normal animals treated with pituitary (anterior lobe) diabetogenic extract. Biochem. J., *39:* 515–536.

ADDENDUM

Since this paper was submitted for publication the article by Moon et al. has come to my attention (''Neoplasms in Rats Treated with Pituitary Growth Hormone. I. Pulmonary and Lymphatic Tissues.'' Cancer Research, *10:* 297–308, May, 1950). In this report 6 of 15 female rats treated for prolonged periods with gradually increasing amounts of growth hormone developed lymphosarcomas of the lung and hyperplasia of peribronchial lymphoid tissue. The lymphatic tissue elsewhere in the body was apparently unchanged by the growth hormone. The above workers used intact female rats of plateaued weight, whereas the animals of the present investigation were young hypophysectomized males. This may explain the differences in lymphoid tissue response.

PLATE 1

EXPLANATION OF FIGURES

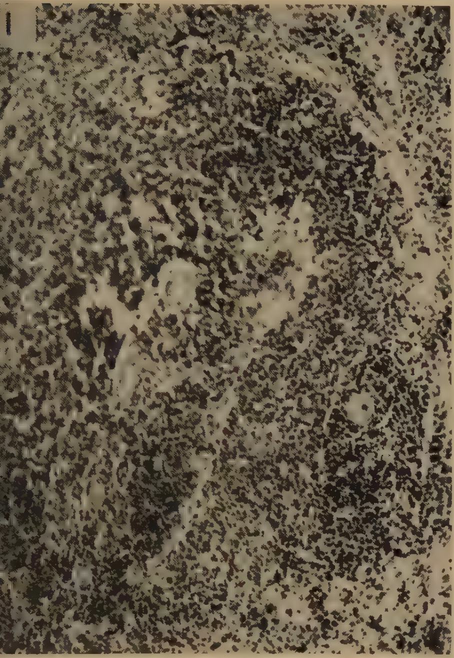
All sections were stained with phloxine methylene blue.

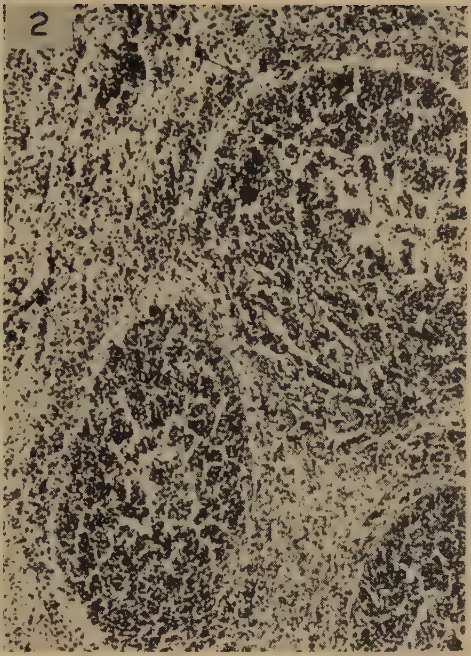
1 Follicle and perifollicular envelope from spleen of fasted control rat. × 150.

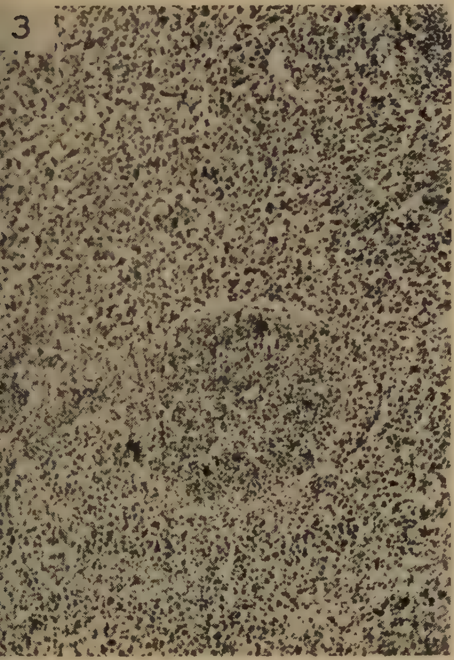
2 Two follicles and perifollicular envelopes from spleen of hypophysectomized rat. Note the diminution in width of the perifollicular envelope and relative lack of cellularity of the pulp. × 150.

3 Splenic follicle from hypophysectomized rat treated with growth hormone. Note blending together of pulp and perifollicular zones and marked increase in cellularity. × 150.

4 Portion of splenic follicle from the spleen of an adrenalectomized rat. Note similarity to figure 3. × 150.


1


2


3

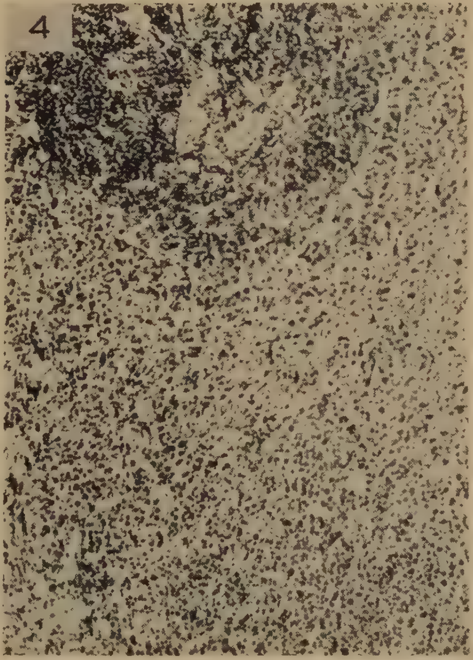

4

PLATE 2

EXPLANATION OF FIGURES

All sections were stained with phloxine methylene blue.

5 High power view of pulp from spleen shown in figure 2, hypophysectomized rat. Note relatively few cells and small size of individual elements. × 600.

6 High power view of pulp from spleen shown in figure 3, hypophysectomized rat treated with growth hormone. Note marked cellularity and large size of individual cells. × 600.

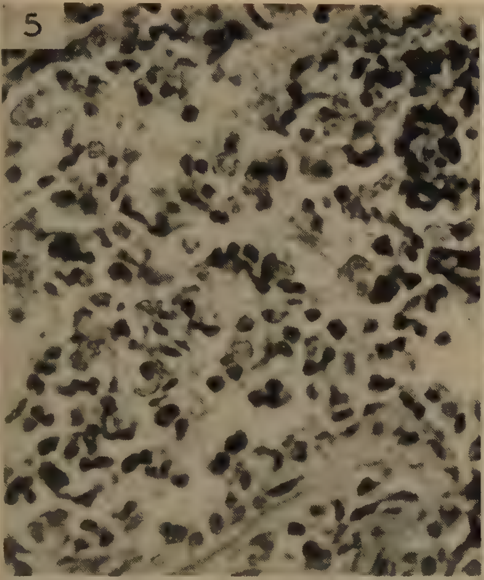
7 Iron stain of section of spleen from fasted control rat. × 11.

8 Iron stain of section of spleen from hypophysectomized rat. × 11. Note decrease in size of organ and relative increase in iron positive material.

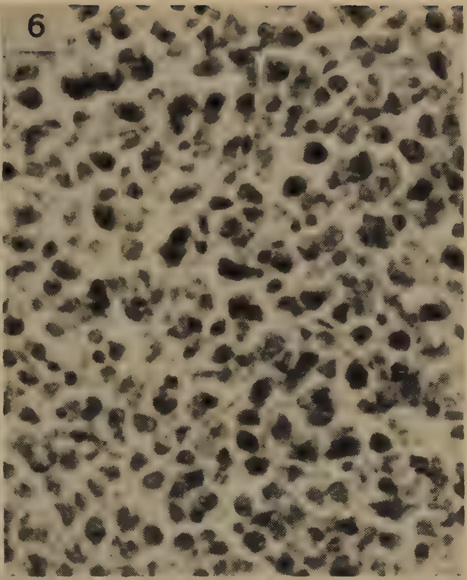
9 Iron stain of section of spleen from hypophysectomized rat treated with growth hormone. × 11. Note increase in size over that seen in figure 8 and almost complete absence of iron-staining material.

10 Iron stain of section of spleen from adrenalectomized rat. × 11. Note increase in size of organ over that seen in figure 7 and approximately the same amount of iron positive material as found in figure 7.

5

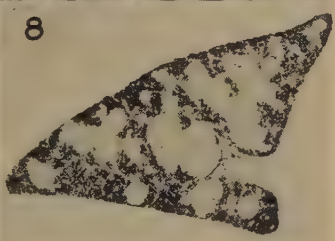


6

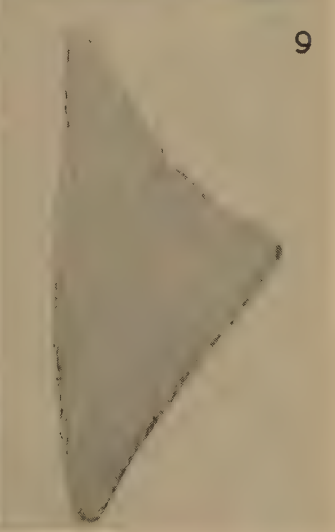


7

8



9



10

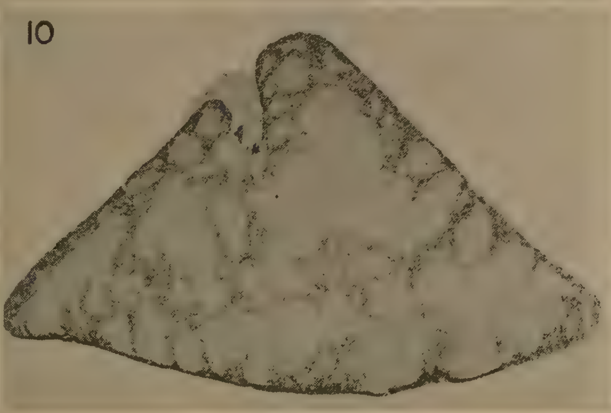


PLATE 3

EXPLANATION OF FIGURES

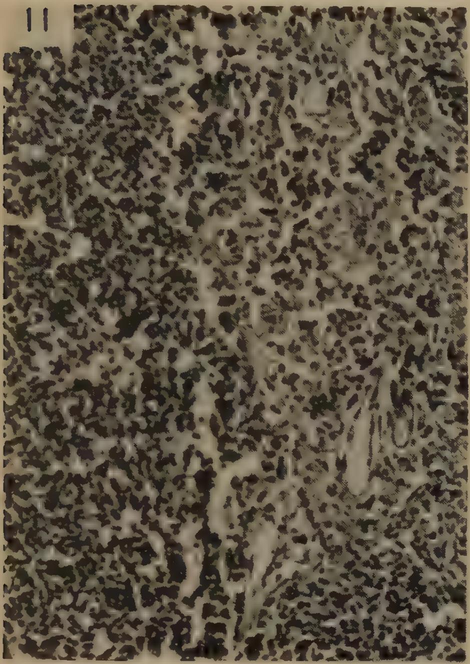
All sections were stained with phloxine methylene blue.

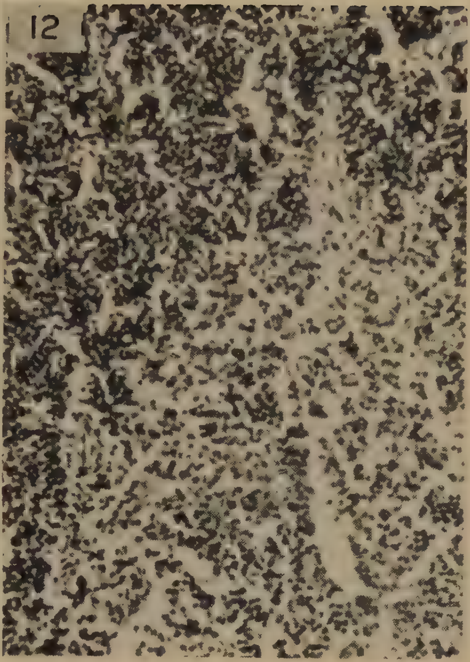
11 Portion of cortex and medulla of thymus from a fasted control rat. × 290.

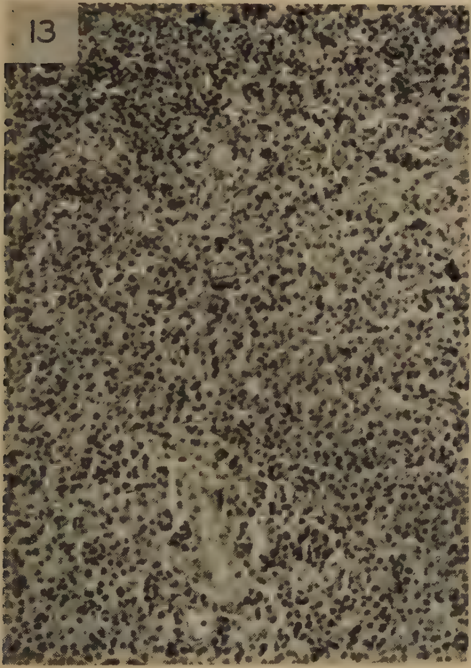
12 Portion of cortex and medulla of thymus from a hypophysectomized rat. × 290.

13 Portion of cortex (in upper part of field) and medulla of thymus from hypophysectomized rat treated with growth hormone. Note increase in size of individual cells. × 290.

14 Portion of cortex and medulla of thymus from adrenalectomized rat. Note increase in cellularity. × 290.


11


12


13

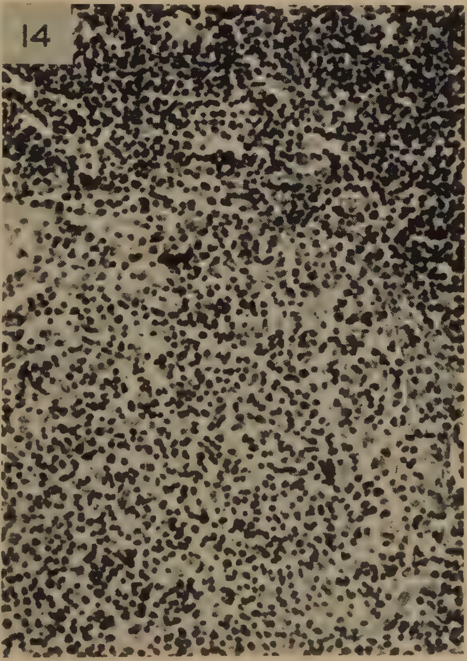

14

PLATE 4

EXPLANATION OF FIGURES

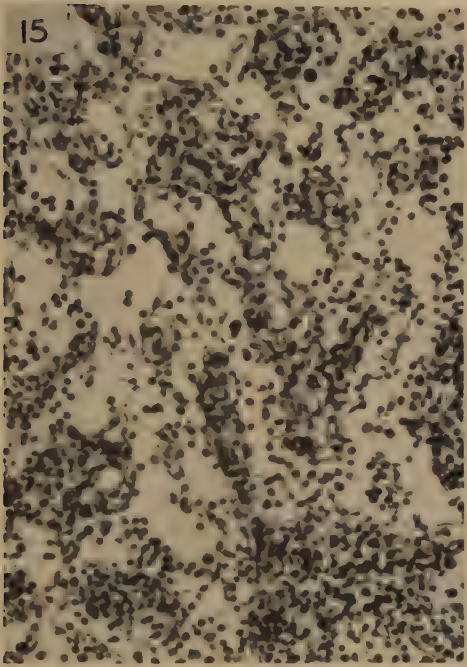
All sections were stained with phloxine methylene blue. Comparable fields of medullary cords were photographed.

15 Medullary cords of lymph node from fasted control rat. × 290.

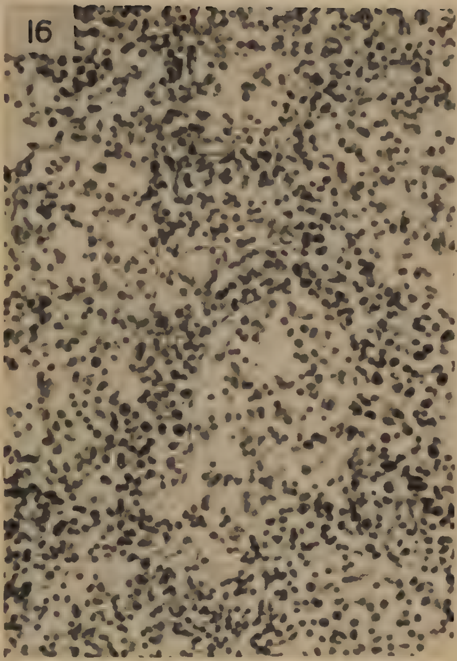
16 Medullary cords of lymph node from hypophysectomized rat. × 290.

17 Medullary cords of lymph node from hypophysectomized rat treated with growth hormone. Note cellularity of cords and size of individual cells. × 290.

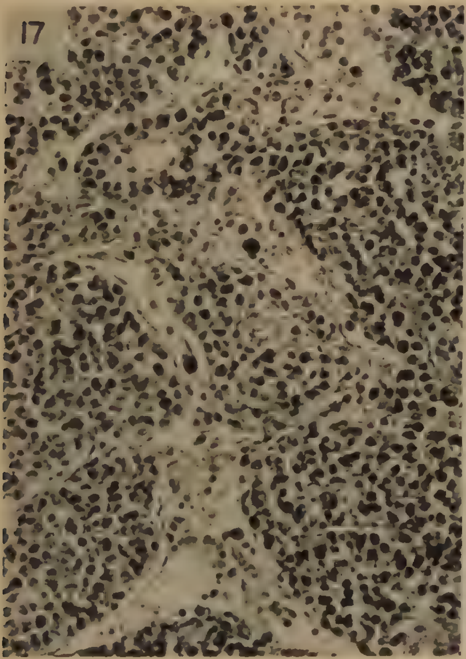
18 Medullary cords of lymph node from adrenalectomized rat. Compare with figure 17. × 290.



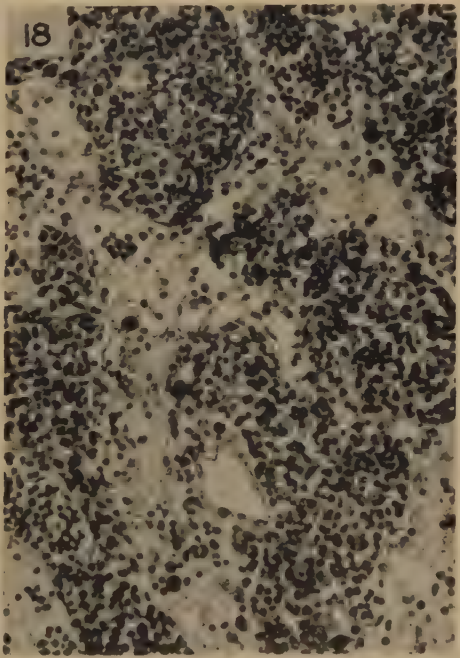
15



16



17



18

A METHOD FOR OPENING AND CLOSING EGGS WITH AN EXPERIMENTAL DEMONSTRATION OF ITS ADVANTAGES

WILHELM HARKMARK AND KNUT GRAHAM
Anatomical Institute, University of Oslo, Norway

EIGHT FIGURES

A handy method for opening and closing eggs is of great importance in experimental embryology for workers using avian material.

Several methods have been described. Hamburger ('34, '42), Rawles ('36), Bueker ('47) and others simply cut a rectangular hole in the shell above the embryo, carefully avoiding any injury to the shell membranes. After previous moistening with saline solution, the shell membranes are removed. When the operation is finished the window is sealed by a coverglass or a piece of cellophane. If desired, the removed eggshell piece is sealed into its original site. Then the egg can be turned in such a position that the blastoderm is covered by untouched shell membrane (Hamburger, '34).

In our opinion this procedure has some obvious disadvantages.

1. In early stages of development albumen often escapes from the egg when the shell membranes are removed.

2. In later stages (three days and more) the extraembryonic vessels are apt to be damaged.

3. Instruments used in removing the shell membranes come in close contact with the egg contents.

4. If the opening in the shell is not placed exactly above the embryo, it is often difficult to bring the latter in position for operation.

The eggshell cap method was described by Paff ('39), and likewise by Price and Fowler ('40). Before them it was used in a similar way by Kuo ('32) and Orr and Windle ('34). In this method the egg is incubated standing on its pointed end. The shell and outer shell membrane above the air cell are removed, and the inner shell membrane is then cleared away. After the operation, the opening in the egg is covered with an eggshell cap made from another egg. The advantage of this method is that there is no danger of injurying the embryo during the opening of the shell. But the removal of the inner shell membrane is beset with the same difficulties as mentioned before. Moreover, the embryo is often not situated just beneath the opening in the shell, but somewhat to one side or the other, thus rendering the embryo inaccessible. The eggshell cap, furthermore, in many instances does not fit well, and during the subsequent incubation the egg contents evaporate to some extent. Consequently, the shell membranes as well as the embryonic membranes become dry and hard, greatly impeding the hatching of the chick.

The difficulties involved in opening the eggs are greatly reduced if the egg contents are allowed to sink and escape from the normal intimate contact with the shell membrane. This was achieved by Fugo and Witschi ('38) in the following manner: A fine hole was bored in the pointed end of the egg, and 2 cm^3 of the albumen was withdrawn. Then an opening in the shell on the uppermost side of the egg could be made without any injury to the blastoderm.

Hillemann ('42) obtained the same advantage in a simpler way, and without any loss of the egg contents. In his method the normal air chamber is obliterated, and a new air cell is formed beneath the inner shell membrane at the uppermost side of the egg. After the operation, the egg is closed with a coverglass, and the air chamber is re-established.

We have used this method for the *opening* of eggs with success in a series of experiments. No candling is necessary before the opening of the egg, and no particular care has to be taken to avoid injury to the embryo.

The *closing* of the eggs after the Hillemann method, however, has proved less satisfactory. When the air chamber is re-established, and the egg contents thus raised up against the coverglass, the yolk mass or the embryonic membranes are inclined to stick to the rough edges of the shell. According to Hillemann, this can be avoided by a quick turning of the egg, so that the coverglass faces downwards. This is possible in early stages of development, but in operations performed on 4–7 day embryos the vascular area is more extensive, lies closer to the shell membranes and consequently, there is greater danger of injuring the embryo.

Better results were obtained when the natural air chamber was not restored.

We found also that the turning of the closed eggs was unnecessary and could be omitted. The eggs were simply covered with a piece of eggshell or a coverglass and not turned at all. In the course of some days the air chamber re-established itself in spite of the artificial air mass at the side of the egg.

Because our technique differs somewhat from that used by Hillemann in some other respects also, a full description of the method is given. Figures 1 to 8 show the different steps in our procedure diagramatically.

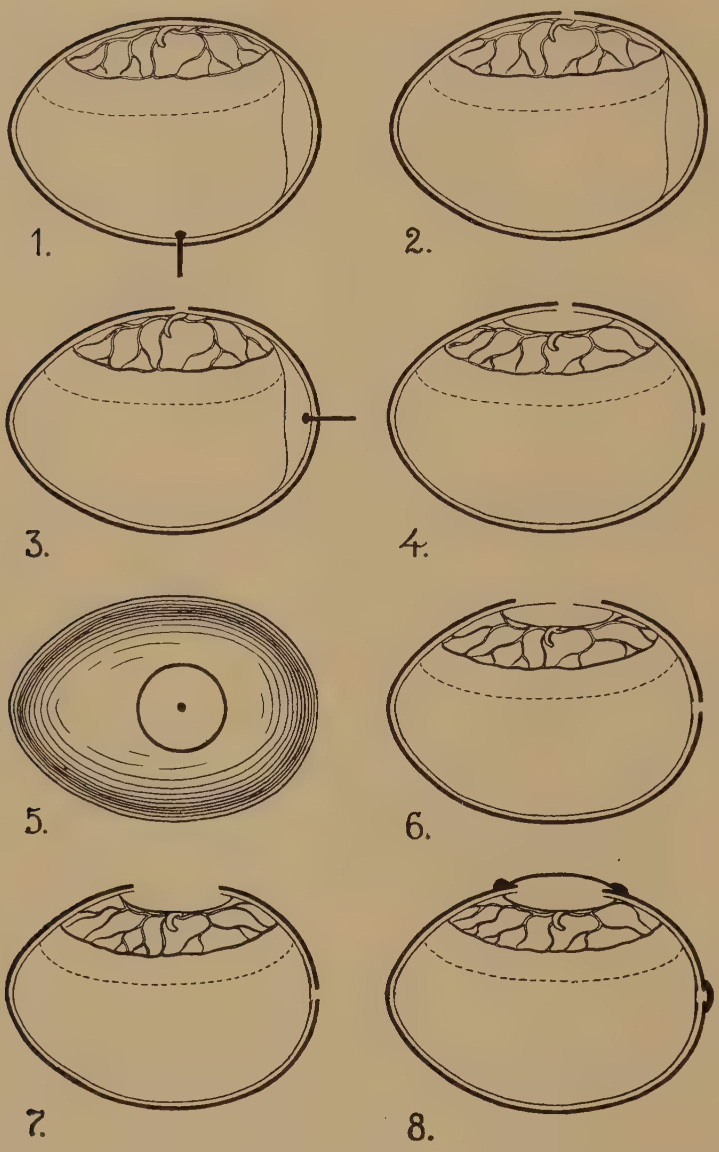
1. With a dental drill a hole is made from below through the shell and shell membranes (fig. 1).

2. The egg is quickly turned so that this opening is uppermost (fig. 2).

3. Another hole is bored in the blunt end of the egg through the shell and outer shell membrane only (fig. 3).

4. Now the air will escape from the air chamber, and a new air cell is formed beneath the inner shell membrane at the uppermost side of the egg. If necessary, air is pressed through this latter opening, for instance with the aid of a Hillemann's air cell cup (fig. 4).

5. A ring of convenient size for a shell window is marked around the hole on the side of the shell. (We use a special stamp for this purpose.) Along this mark a groove is made in



Figs. 1–8 Diagrams of the different steps in the method described. In figure 5 the egg is seen from above. The other figures demonstrate the relation between the egg contents and the egg walls in an egg of approximately 4 days incubation. Half of the shell and shell membranes are removed, leaving the egg contents in place and visible. See text.

the shell with a carborundum disc run by an electric motor. If the shell is not penetrated by the disc, the shell dust easily can be wiped away with alcohol (fig. 5).

6. The shell piece is lifted away (fig. 6).

7. The shell membranes are removed. Now the embryo in most instances presents itself for operation. The air chamber, as Hillemann points out, permits the egg to be tilted over a wide range of angles to bring the embryo in optimum position for operation (fig. 7).

8. Following the operation, the opening in the shell is closed with a coverglass or a watch glass and sealed with wax (fig. 8).

If the embryo is to live for some hours or a few days only, it is sufficient to cover the opening with an unsealed piece of egg shell, from which the membranes have been removed, the piece dipped in alcohol and sterilized in a flame. Finally the hole in the blunt end of the egg may be sealed with wax, but the results are equally good when this hole is left open.

In order to get an idea of the value of this modification of Hillemann's procedure, the methods were tested in hatching experiments with normal eggs as controls. The eggs in these experiments were opened and then immediately closed. No operations were performed on the embryos themselves.

In a first set of experiments 100 fertilized eggs on the 4th day of incubation were selected by candling and divided into 4 groups to test the effect of opening and closing (table 1). The eggs in the first group were incubated in the regular manner. In the second group the eggs were not turned. In the third and fourth groups the eggs were opened and closed with coverglasses, but the air cells were not re-established. The eggs in the third group were incubated in unaltered position, in the fourth group they were turned regularly during the subsequent incubation.

As will be seen there is little difference between the three first groups. In the last group, turned at regular intervals, however, only one chick hatched.

A second set of experiments was carried out to study the effect of turning at different stages of development (table 2). In this series 50 fertilized eggs were opened on the second day and 50 eggs on the 5th day of incubation. Each time half of the eggs were treated according to the Hillemann method (groups 4 and 5), the rest were closed after the modification of this method described in this paper (groups 2 and 3). Group 1 is a control group.

TABLE 1

The effect of opening and turning eggs upon the hatching of chick embryos

GROUPS	1 UNOPENED, TURNED	2 UNOPENED, NOT TURNED	3 OPENED, CLOSED, NOT TURNED	4 OPENED, CLOSED, TURNED
	(Control gr.)	*(Control gr.)*		
Numbers of eggs	25	25	25	25
Day of opening			5th	5th
Alive on 5th day	25	25	25	25
Alive on 10th day	24	22	22	10
Alive on 15th day	24	22	21	9
Hatched chicks	15	12	14	1
			Air cell not restored	

TABLE 2

The effect of turning at different stages of incubation

GROUPS	1 UNOPENED, TURNED	2 OPENED, CLOSED, NOT TURNED	3 OPENED, CLOSED, NOT TURNED	4 OPENED, CLOSED, TURNED	5 OPENED, CLOSED, TURNED
	(Control gr.)				
Number of eggs	25	25	25	25	25
Day of opening		2nd	5th	2nd	5th
Alive on 5th day	25	25	25	25	25
Alive on 10th day	25	24	21	17	21
Alive on 15th day	18	24	20	14	6
Hatched chicks	15	19	10	1	0
		Air cell not restored		Air cell restored	

As compared with the corresponding group (3) in the previous series (table 1) the number of hatched chicks in group 3 was small, but still greater than in any of the groups containing eggs which were turned after the closing.

SUMMARY AND CONCLUSIONS

As in Hillemann's original method, the air cell in the present method is obliterated before the opening of the egg, and a new air cell is formed beneath the inner shell membrane at the uppermost side of the egg.

The essential points in the method suggested here are:

1. The natural air cell is not re-established.
2. The eggs are not turned during the subsequent incubation.

The two methods have been compared in hatching experiments with normal eggs as controls. The experiments demonstrate that whether the air cell is re-established or not, turning of the closed egg during incubation is unnecessary, or even injurious.

On the other hand good results are obtained when eggs are not turned after the closing. After hatching chicks from the unturned eggs in every respect develop as well as controls.

Our method has certain advantages over that of Hillemann, particularly for keeping the embryos alive for several days. In addition it is simpler and easier to perform.

LITERATURE CITED

BUEKER, E. D. 1947 Limb ablation experiments on the embryonic chick and its effect as observed on the mature nervous system. Anat. Rec., *97:* 157–174.

FUGO, N. W., AND E. WITSCHI 1938 Removal of the hypophyseal primordium in the chick during the second day of incubation. Acta Biologica Latvica, *8:* 73–79.

HAMBURGER, V. 1934 The effects of wing bud extirpation on the development of the central nervous system in chick embryos. J. Exp. Zool., *68:* 449–492.

——— 1942 A manual of experimental embryology. The University of Chicago Press. Chicago, p. 204.

HILLEMANN, H. H. 1942 A method and apparatus for opening and closing eggs which will permit turning at regular intervals during subsequent incubation. Anat. Rec.., *84:* 331–336.

KUO, Z. Y. 1932 Ontogeny of embryonic behavior in aves. 1. The cronology and general nature of the behavior of the chick embryo. J. Exp. Zool., *61:* 395–430.

ORR, D. W., AND W. F. WINDLE 1934 The development of behavior in chick embryos: The appearance of somatic movements. J. Comp. Neur., *60:* 271–285.

PAFF, G. H. 1939 The action of colchicine upon the 48-hour chick embryo. Am. J. Anat., *64:* 331–340.

PRICE, J. W, AND E. V. FOWLER 1940 Eggshell cap method of incubating chick embryos. Science, *91:* 271

RAWLES, M. E. 1936 A study in the localization of organ-forming areas in the chick blastoderm of the head-process stage. J. Exp. Zool., *72:* 271–315.

VARIATIONS OF THE TENDONS OF INSERTION OF THE ABDUCTOR POLLICIS LONGUS AND THE EXTENSOR POLLICIS BREVIS

ARTHUR H. STEIN, JR.
Department of Anatomy, Washington University, St. Louis

FIVE FIGURES

A form of stenosing tendovaginitis which involves the osteofibrous canal occupied by the tendons of the abductor pollicis longus and the extensor pollicis brevis muscles, frequently known as de Quervain's disease, usually requires surgery. Bunnell ('48) in his book "Surgery of the Hand," mentions that in 12 of his 22 cases of de Quervain's disease accessory tendons were present, and that a review of the standard texts of anatomy led him to believe that accessory tendons in this region were relatively rare.

Standard texts of anatomy (Cunningham, '40; Morris, '42; and Gray, '48) do state that accessory tendons of insertion, particularly for the abductor pollicis longus muscle, have been observed. The variations are well described in "Traité D'Anatomie Humaine" by Poirier (1896) and "Traité Des Variations Du Système Musculaire De L'Homme" by LeDouble (1897). The absence or doubling of the tendon of the extensor pollicis brevis is noted as well as the fact that multiple accessory tendons of the abductor pollicis longus may be found. The tendons of the latter muscle have been found to insert not only into the base of the first metacarpal, as is usually described, but also into the trapezium or into the muscles and fascia of the thenar eminence. The only record of the incidence of variations of the abductor pollicis longus is by Wood (Poirier, 1896) who found an accessory tendon inserted into the thenar muscles in 7 of 36 bodies examined. Approximately one-half of the cases of de Quervain's disease seen in Barnes Hospital during the past three years, presented variations of the tendons. Thus, the anatomy in an unselected series of wrists has been examined in order to determine the incidence of these variations.

PROBLEM

The usual site of insertion stated for the tendon of the abductor pollicis longus muscle is into the radial side of the base of the first metacarpal bone. This tendon passes through the first osteofibrous canal on the lateral surface of the distal extremity of the radius. The canal is formed by the dorsal carpal ligament and that part of the radius between its prominences to which the ligament is attached. Passing through the same osteofibrous canal is the tendon of the extensor pollicis brevis muscle, and it is usually described as inserting into the base of the proximal phalanx of the thumb. The osteofibrous canal is approximately 1 cm in length, but is continuous both proximally and distally with a synovial tendon sheath surrounding both of these tendons. This tendon sheath extends about 1 cm proximal to and 2.5 cm distal from the dorsal carpal ligament.

Considering the above as the normal anatomical arrangement, interest was directed toward the following major variations: (1) presence of accessory tendons and their sites of insertion; (2) absence of one (or more) of the tendons; (3) bilateral asymmetry; and (4) whether or not both tendons occupy the same osteofibrous canal.

MATERIAL AND METHOD

The material used in this study was the series of cadavers dissected by the first-year class of Washington University School of Medicine in their study of gross anatomy. Each wrist was carefully dissected by the writer following skinning of the forearm and hand by the student. A total of 42 cadavers or 84 wrists were dissected. This group of cadavers

MAR 2 3 1952

was composed of 19 Negro males, 11 White males, 8 white females, and 4 Negro females, ranging in age from 41 to 90 years.

OBSERVATIONS

Of the 84 wrists dissected 57 (68%) showed one or more accessory tendons of insertion for the abductor pollicis longus, and these were demonstrable just proximal to, within, or just distal to the osteofibrous canal. In comparing the incidence of accessory tendons in the male and female, and in the Negro race and White race, a significant difference was not apparent. In the Negro cadavers 31 (67%) of 46 wrists showed accessory tendons for the abductor pollicis longus; whereas in the White race 26 (68%) of 38 wrists showed accessory tendons. In the male cadavers 41 (68%) of 60 wrists showed accessory tendons; and in the female cadavers the ratio was 16 (67%) in 24 wrists. In 24 dissections the accessory tendons of the abductor pollicis longus inserted into the muscles or fascia of the thenar eminence, while 24 other accessory tendons were found to insert into the trapezium. In 11 cases the accessory tendon inserted both into the trapezium and into the muscles and fascia of the thenar eminence. In two of the 57 wrists there were two accessory tendons for the abductor pollicis longus present, thus accounting for 59 accessory tendons. It was also noted that when accessory tendons were present they were joined to the main tendon for a short distance by a web of synovium. Many of the accessory tendons were of the same size as the "normal" tendon, which was considered to be the one inserting into the base of the first metacarpal.

Of the 84 wrists studied 7 showed doubling of the tendon of the extensor pollicis brevis, and in three (4%) of these the double tendon was evident just proximal to, within, or just distal to the osteofibrous canal. In 5 of these cases the accessory tendon was attached to the tendon of the extensor pollicis longus. In 6 wrists a distinct extensor pollicis brevis tendon could not be identified; however, in these cases a large single tendon was seen to occupy the canal. From this single tendon, at the base of the metacarpal, a slip of tendon extended to insert into the base of the proximal phalanx of the thumb. This large tendon also frequently gave rise to slips of tendon which inserted into the trapezium or into the muscles and fascia of the thenar eminence. Two examples of this latter condition are cited:

> Cadaver 17-R, White female, age 66 years.
>
> The tendon of the extensor pollicis brevis was normal on the right, absent on the left. The tendon of the abductor pollicis longus was double on both sides in the osteofibrous canal. The division was about $\frac{1}{4}$ and $\frac{3}{4}$. The larger portion inserted into the base of the first metacarpal on both sides. The smaller portion inserted into the fascia over the thenar muscles on the right side, but into the base of the proximal phalanx of the thumb on the left side.
>
> Cadaver C-262, White male, age 74 years.
>
> On both sides only a single large tendon was found in the osteofibrous canal. On the right this tendon gave rise to three separate tendons 1 cm distal to the canal, and these three inserted into the base of the proximal phalanx of the thumb, into the base of the first metacarpal, and into the trapezium, respectively. On the left only two tendon slips extended from the large tendon; the one to the base of the proximal phalanx was missing. However, on the left side a slip of tendon extending from the base of the proximal phalanx was present. Its proximal attachment was indefinite, appearing to be attenuated to the distal end of the radius.

In the three wrists, in which the tendon of the extensor pollicis brevis was found to be double in the region of the osteofibrous canal, the tendon of the abductor pollicis longus was also double in the same region. In 7 cadavers the tendon of the extensor pollicis brevis showed a major difference between the right and left sides, and this was also true for the tendon of the abductor pollicis longus in 7 cadavers.

In 9 of the 84 wrists the tendons of the extensor pollicis brevis and the abductor pollicis longus were found in separate osteofibrous canals. The division of this usually single canal was made by a bony ridge of the radius and an associated fibrous septum.

CONCLUSIONS

The wrists of 42 cadavers have been dissected in order to determine the frequency of accessory tendons of insertion of the extensor pollicis brevis and abductor pollicis longus muscles. In these 84 wrists, accessory tendons of the extensor pollicis brevis were found three times (4%) and of the abductor pollicis longus 57 times (68%) in the region where these two tendons pass through the same osteofibrous canal. It would appear then, that in addition to the usually described tendon of insertion of the abductor pollicis longus (into the base of the first metacarpal) there is present an accssory tendon, demonstrable within, just proximal to or just distal to the osteofibrous canal, which inserts into the trapezium or into the muscles and fascia of the thenar eminence. Although 84 wrists do not comprise a large series there is no evidence of a race or sex difference since an accessory tendon of the abductor pollicis longus is present in either 67% or 68% of each group of Whites, Negroes, males and females.

Acknowledgment is made to Dr. Mildred Trotter for her suggestions during the preparation of this manuscript.

LITERATURE CITED

BUNNELL, S. 1948 Surgery of the Hand. 2nd ed. J. B. Lippincott Co.
LEDOUBLE, A. F. 1897 Traité Des Variations du Système Musculaire. Tome Second. Schleicher Freres, Editeurs, Paris.
POIRIER, P. 1896 Traité D'Anatomie Humaine. Tome 2. Masson et Cie, Editeurs, Paris.

PLATE 1

EXPLANATION OF FIGURES

In the diagrams each cross-hatched square represents the osteofibrous canal. The upper and smaller of the two tendons is the extensor pollicis brevis, and the larger is the abductor pollicis longus.

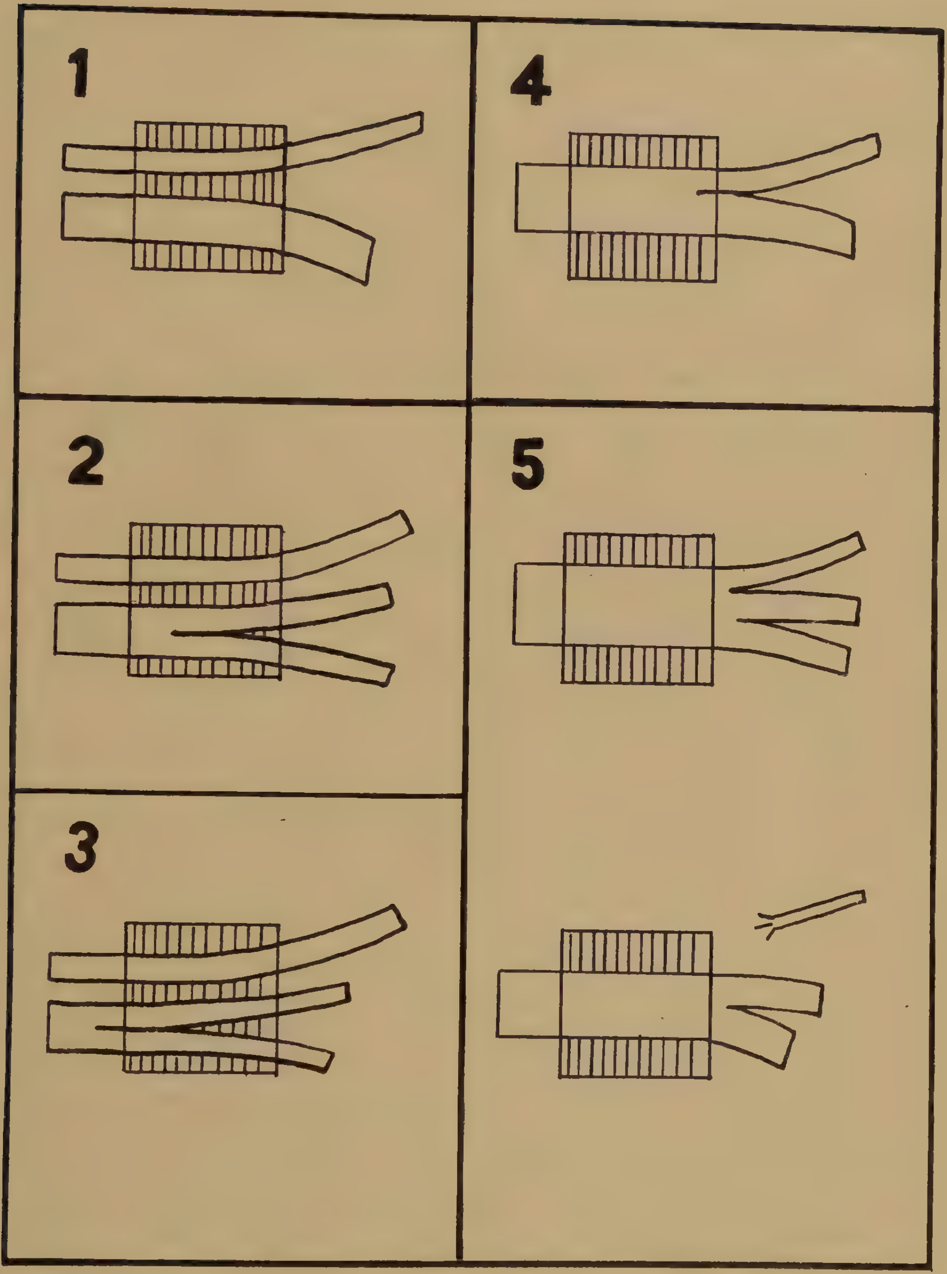
1 Represents the arrangement that has been considered to be normal, viz., two tendons passing through the common osteofibrous canal.

2 Shows division of the tendon of the abductor pollicis longus in the canal, the two parts inserting into the base of the first metacarpal and into the trapezium.

3 Shows division of the abductor pollicis longus proximal to the canal; one part going to the first metacarpal and the other to the muscles and fascia of the thenar eminence.

4 Shows the arrangement in the left wrist of cadaver 17-R. The tendon of the extensor pollicis brevis is absent. The abductor pollicis longus was divided into two parts, the upper division passing to insert into the base of the proximal phalanx of the thumb. and the lower division into the first metacarpal.

5 Arrangement in the wrists of cadaver C-262. The upper figure represents the right wrist with the division of the single tendon into three separate parts each of which has its own site of insertion (see text). The lower figure represents the left wrist showng the slip of tendon attenuated from the distal end of the radius and inserting into the base of the proximal phalanx of the thumb as described in the text.



THE DEMONSTRATION OF THE INTERCAPILLARY SPACE OF THE HUMAN RENAL GLOMERULUS

J. F. A. McMANUS, C. H. LUPTON, JR., AND L. S. GRAHAM, JR.
School of Pathology, Department of Medicine, University of Virginia, Charlottesville, Virginia

TWO FIGURES

The recent publication of Ritter and Oleson ('50) describes the combined application of the Hale ('46) stain for acid polysaccharides and the periodic acid-Schiff's reagent method for carbohydrates (McManus, '46; Lillie, '47; Hotchkiss, '48). Some carbohydrates are colored blue-green and others red-purple. The procedure is taken to demonstrate "polysaccharides of two types." The histochemical specificity of this method is considered only briefly here. This article describes the demonstration of the intercapillary space (McManus, '48b) by the Ritter-Oleson ('50) method. The accentuation of the intercapillary space (McManus, '48a, b, '50) in diabetes mellitus is used to define further the existence of the structure.

Zimmerman ('33) described the mesangium as a connective tissue framework of the glomerulus. Kimmelstiel and Wilson ('36) and Goormaghtigh ('42) have used the concept of mesangium in explaining disease processes. Since the "mesangium" appears lacking in formed elements, McManus ('48b) has suggested the term "intercapillary space." Bell ('46) and others doubt the existence of any such structure.

The illustration by Ritter and Oleson ('50) of the rat renal glomerulus appeared to stain the intercapillary space differentially. The central portion of the glomerulus is colored red, the basement membrane of the capillary loops is blue-green. The method was applied to human autopsy material with minor modifications as described.

MATERIALS AND METHODS

A group of human autopsy cases were studied. Blocks of kidney were fixed for less than 24 hours, usually 18–20 hours, in alcohol-formalin (Ritter and Oleson, '47). The blocks were subsequently handled as described by Ritter and Oleson ('50) with the exception that no pyronine stain was used en bloc. Zenker formol fixation is quite satisfactory. Paraffin sections at 6 μ brought through xylene and alcohols to distilled water were handled as follows:

1. Stained for 10 minutes in equal parts of:
    (a) Dialyzed iron.
    (b) Molar acetic acid.
2. Washed well in distilled water.
3. Stained 10 minutes in equal parts of:
    0.02 M potassium ferrocyanide, freshly made up.
    0.14 M HCl.
4. Washed well in tap and distilled water.
5. One-half per cent aqueous periodic acid — 5 minutes.
6. Rinsed in distilled water.
7. Schiff's reagent 15 minutes, sulfurous acid rinses, etc., as in the PAS method previously described in detail (McManus, '48b, c).
8. After washing well in tap water the sections were dehydrated in graded alcohols, cleared in xylene and mounted in balsam.

No reducing rinse (Hotchkiss, 48) was used for reasons previously given (McManus, '48c).

RESULTS

The method of Ritter and Oleson ('50) gives by all odds the most striking and convincing demonstration of the intercapillary space of the human renal glomerulus. The intercapillary space is colored red to purple. The capillary loops of the glomerulus are colored blue (fig. 1). Sometimes a fine red or purple line can be seen beneath the blue of the capillary loop.

In suitable sections it can be seen that wherever the capillary loops come together the junction is a red-colored or red-outlined area. This feature is best seen in the peripheral portions of the glomerulus. In the more central areas of the glomerulus and especially at its attachment to the arterioles some of the red material in the glomerulus is continuous with the intercellular substance of the arterioles. When an appropriate section is studied, it can be seen that this arteriolar and glomerular red material is continuous with that in the junction points of the peripheral loops. This feature is best made out in figure 2.

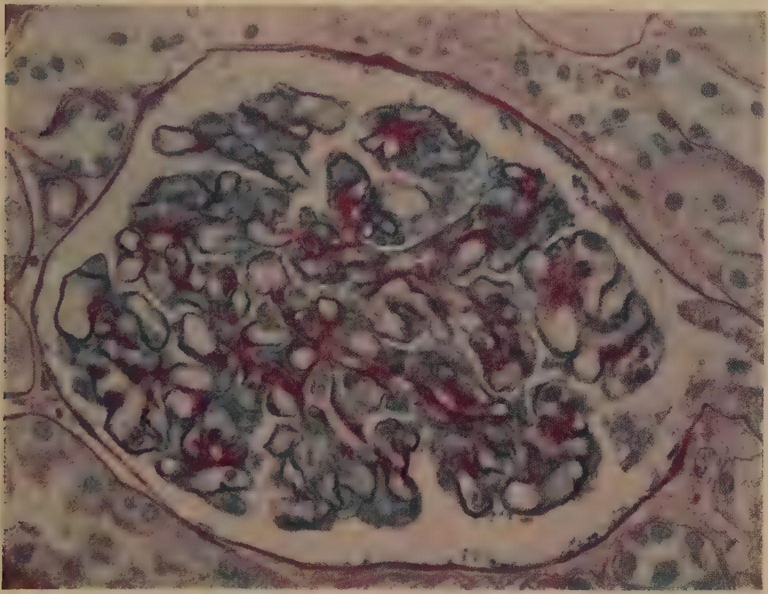


Fig. 1 Normal glomerulus of a human kidney colored with the Ritter and Oleson ('50) method. The glomerular intercapillary space is colored red. The glomerular basement membrane over the capillaries is colored blue. Kodachrome × 250.

The visceral glomerular basement membrane is colored blue unlike the parietal or tubular basement membranes or the intercapillary space, all of which are red. This blue coloration allows the easy identification of the capillary loop basement membrane and the demonstration of red-colored intercapillary space. In the cases of intercapillary glomerulosclerosis, the abnormal glomerular material colors red and is present in the intercapillary space (fig. 2). Stages of the development of the glomerular lesion from normal to hyaline nodules can be observed in different glomeruli.

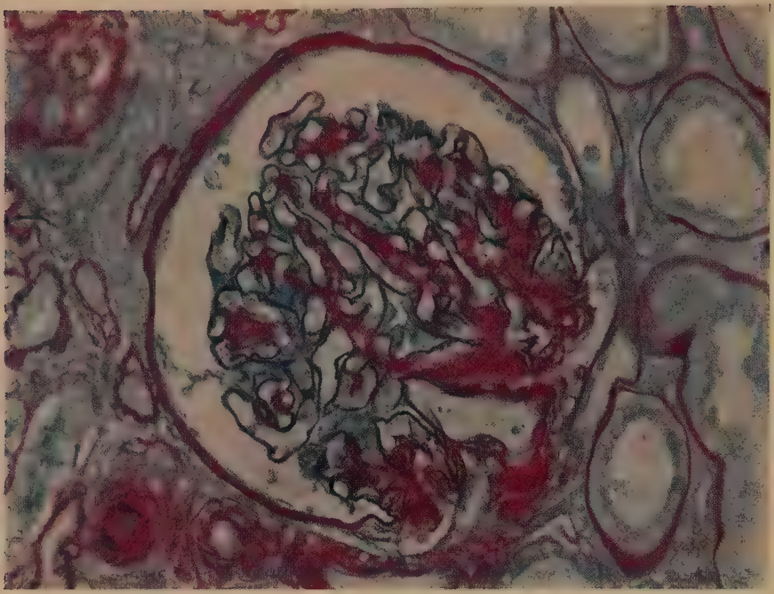


Fig. 2 Human renal glomerulus colored as in figure 1 from a case of intercapillary glomerulosclerosis. The abnormal glomerular material is colored red and accentuates the intercapillary space. Kodachrome × 190.

DISCUSSION

The differential staining of the glomerular intercapillary space by the method of Ritter and Oleson ('50) makes its existence quite obvious. The method should prove of considerable value in the study of the kidney and its diseases. A beginning has been made in the present report by the descriptions of the normal condition and of the changes in intercapillary glomerulosclerosis.

The intercapillary space or area (Mesangium of Zimmerman, axial space) conforms in all regards to the previous descriptions (McManus, '48b, '50). It is an axis continuous with the vascular pole of the glomerulus. The capillaries curve and course around this framework or space. The individual capillary loop has an external basement membrane, coloring blue with the Ritter and Oleson ('50) method, across which filtration occurs. This external basement membrane of the capillary loop appears to be the basement membrane of Bowman's capsule invaginated by the tuft of capillaries forming the glomerulus.

The reflection of the visceral epithelium of Bowman's capsule and its basement membrane from capillary to capillary leaves the intercapillary space which colors red or is outlined in red by the Ritter and Oleson ('50) method. There is no recognizable internal basement membrane of the capillary loops, the external basement membrane reflecting from loop to loop, being actually the basement membrane of Bowman's capsule. Favoring this interpretation is the absence of an internal basement mambrane with either the periodic acid method (McManus, '48b, c) or the Ritter and Oleson ('50) method at the junction points described above.

The present study gives some information about the nature of the material normally present in the intercapillary space. Ritter and Oleson ('50) describe a coloring of "fibres of the glomerulus" by their method. It is highly probable that they are describing the intercapillary space.

If the material in the intercapillary space is of fibrous nature it is peculiar that it gives no coloring for collagen or any staining by the majority of the silver methods for reticulin. The only silver reticulin method which consistently colors glomerular structures is Wilder's stain ('35). As pointed out by Olcott ('48) the method of Wilder colors glomerular basement membrane. Wilder's reticulin stain is unreliable for definitive studies. It is our impression that the intercapillary space in the normal human renal glomerulus contains carbohydrate material, the nature of which is not further advanced by the present study.

The histochemical specificity of the Ritter and Oleson ('50) method "for two polysaccharides" is not suggested in this report. It is to be questioned since many nuclei of tubular cells color by the acid polysaccharide part of the method as used here. The method is discussed as an extremely useful histological procedure for the study of the structure of the human renal glomerulus.

SUMMARY AND CONCLUSIONS

The method of Ritter and Oleson ('50) combining the Hale ('46) procedure for acid polysaccharides and the periodic acid-Schiff's reagent method (McManus, '48b, c) for carbohydrates has been applied to the study of human kidneys.

A selective staining of the intercapillary space of the human renal glomerulus is obtained.

The abnormal glomerular material of intercapillary glomerulosclerosis infiltrates and accentuates the intercapillary space.

The method of Ritter and Oleson ('50) is a valuable histological stain for the study of the human kidney. Its histochemical specificity for acid polysaccharides is questioned.

Photomicrographs by Miss Anne Russel.

REFERENCES

Bell, E. T. 1946 Renal Diseases. pp. 434. Lea and Febiger, Philadelphia.

Goormaghtigh, N. 1941 La doctrine de la cellule musculaire, afibrillaire, endocrine en pathologie humaine. 2. Le rein de l'eclampsie puerpale. Bull. Acad. roy. de Belgique, *7:* S. 6, 194–216.

Hale, C. W. 1946 Histochemical demonstration of acid polysaccharides in animal tissues. Nature, London, *157:* 802.

Hotchkiss, R. D. 1948 A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem., *16:* 131–141.

Kimmelstiel, P., and C. Wilson 1936 Intercapillary lesions in the glomeruli of the kidney. Am. J. Path., *12:* 83–97.

Lillie, R. D. 1947 Studies on the preservation and histological demonstration of glycogen. Bull. Internat. Association Med. Museums, *27:* 23–61.

McManus, J. F. A. 1946 Histological demonstration of mucin after periodic acid. Nature, London, *158:* 202.

——— 1948a The periodic acid routine applied to the kidney. Am. J. Path., *24:* 643–653.

——— 1948b Structure of the glomerulus of the human kidney. Am. J. Path., *24:* 1259–1269.

——— 1948c Histological and histochemical uses of periodic acid. Stain Tech., *23:* 99–108.

——— 1950 Medical Diseases of the Kidney. pp. 176. Lea and Febiger, Philadelphia.

OLCOTT, C. 1948 Experimental Argyrosis. IV. Morphologic changes in the experimental animal. Am. J. Path., *24:* 813–834.

RITTER, H. B., AND J. J. OLESON 1947 A peroxidase reaction in paraffin sections. Arch. Path., *43:* 330–332.

——————— 1950 Combined histochemical staining of acid polysaccharides and 1, 2 glycol groupings in paraffin sections of rat tissues. Am. J. Path., *26:* 639–645.

WILDER, H. C. 1935 An improved technique for silver impregnation of reticulum fibers. Am. J. Path., *11:* 817–820.

ZIMMERMAN, K. W. 1933 Über den bau des glomerulus der saungerniere, weitere mitteilungen. Ztschr. f. mikr.-anat. Forsch., *32:* 176–278.

THE CAPILLARY SYSTEM OF THE DOG'S SPLEEN[1]

THADDEUS H. DOGGETT[2]
Department of Anatomy, Tulane University, New Orleans, Louisiana

TEN FIGURES

INTRODUCTION

A venous capillary system uniting the arterial capillaries with the venous sinuses has not been conclusively demonstrated previously in the mammalian spleen. The present study is based upon a method whereby the entire capillary system is demonstrable in ordinary histologic sections, as shown in the accompanying photographs, thus making it possible to trace the continuity of capillaries and sinuses.

The finding in ordinary histologic preparations of great numbers of erythrocytes as well as injected and transfused materials in the pulp spaces has been the main factor supporting the "open circulation" hypothesis. Present hypotheses recognizing the contractile properties of the spleen indicate that a study of the pulp spaces under varying degrees of splenic contraction is necessary for further knowledge of the vascular terminals. A consideration of the part played by the venous capillaries during physiologic contraction and distention of the spleen will be dealt with in the light of findings presented here.

---

[1] Based on a thesis submitted to the Department of Anatomy of the Graduate School of Tulane University in partial fulfillment of the requirements for the degree of Master of Science, May 1, 1950.

[2] The author wishes to acknowledge his appreciation to Drs. T. Snook, N. L. Hoerr, R. Baillif, and S. R. Bruesch for their helpful criticisms in the preparation of this manuscript.

Frequent failure to show in histologic sections any trace of venous capillaries has strengthened the concept of an open circulation supported by many investigators. The current interpretation has shifted to combine the concepts of both open and closed systems of blood channels, but a capillary system uniting arterial capillaries and sinuses is not included in the commonly accepted descriptions. Methods other than those employing histologic sections have been devised by McNee ('31) and Knisely ('36), but conclusive results bearing on the question of venous capillaries have not been obtained. Snook ('44, '50) has shown, in preparations stained for reticular tissue, evidence of direct connections of arterioles with sinuses in the guinea-pig spleen, and Herrlinger ('49) described the same relationship in interfollicular areas of the human spleen. Björkman ('47) recently has shown arterial capillaries opening directly into sinuses in the rabbit spleen. By the use of Snook's reticular stain he has shown in isolated instances the same vascular pattern in the human spleen. He did not describe a venous capillary link in the vascular pathway.

Various injection methods have been used by Helly ('03), Thoma ('24), Mall ('00), McNee ('31), MacNeal ('26) and others. Helly performed many elaborate transfusion experiments using defibrinated chicken blood, frog and salamander blood, and Berlin-blue gelatin masses; he also transfused the spleen with fixing solution. Thoma used a two-solution injection fluid in hope that the particles of dye and the fluid might be separated and give some clue as to the blood course. McNee injected yeast cells into the splenic artery and Mall used asphalt granules. Recent injection experiments utilizing starch grains have been performed by Björkman who found that the larger grains were retained within the sinuses. He concluded that the sinus wall acts as a filter to separate the plasma and red cells.

A thorough description of the arterial capillaries has been given by Robinson ('26) who reviewed the work of Thoma and Mall and added his own observations. He described more fully than previous authors the funnel-shaped dilation of the arterial capillary endings within the pulp cords.

Mall observed numerous capillaries in the distended spleen but was unable to trace their continuity with other channels. Distended spleens were also studied by MacNeal who perfused the organ with a fixative through both the lienal artery and vein. He could find no capillary connection to the sinuses and supported the open circulation theory. Weidenreich ('01) studying the dog spleen observed a capillary entering a sinus; he did not call it a venous capillary but described its origin from an arterial capillary.

Roy (1880–1882) and Barcroft and Stephens ('27) have shown the nature and extent to which the spleen distends and contracts *in vivo*. The contractions, which take place rhythmically, are unrelated to blood pressure and respiration and cease when the nerves to the spleen are cut. Mall reported hemorrhagic lesions in the spleen that resembled infarction after partial denervation of the organ. Histologic sections from the lesions revealed large quantities of blood in the pulp spaces, but no venous capillaries were reported.

McNee ('31) studied the splenic circulation by examination of the organ in the living mouse, but he did not describe a venous capillary link in the system. Knisely ('34, '36) devised a more refined method of observation of the spleen in the living mouse and reported arteriolar connections with sinuses. MacKenzie, Whipple and Wintersteiner ('41) using the same method, reported conflicting results. Recently Peck and Hoerr ('51a, b) have explained the discrepancies and confirmed Knisely's work, but none of these workers described venous capillaries apart from the arterial capillaries and the sinuses.

MATERIALS AND METHOD

Because of the great variation in size which has been observed in the dog spleen and of its ability to distend and contract, this animal was chosen as the source of material. Three spleens were studied. One spleen had been allowed to contract after maximal distention, one was moderately distended and the third was maximally distended. Sections for microscopic study were cut at 10 μ in series from representative blocks, and stained with hematoxylin and eosin.

Distention was produced by pumping 10% formalin into the lienal vein. The vessels were promptly ligated to prevent escape of fluid.

When material is injected into the lienal vein it passes directly into the sinuses of the spleen and readily gains access to the surrounding pulp spaces and reticular meshes. This was seen on several occasions in the course of this study with the use of India ink. No damage to the sinus wall was observed in the histologic examination. If fixing fluid is injected it also gains access to the pulp meshes, but because of its incompressibility, the fluid distends the meshes during injection and prevents contraction at fixation. The preservation and demonstration of capillaries in this type of preparation are dependent on the elasticity of fresh splenic tissue. Tissue examined by this method more than an hour after death was found to have lost the elasticity necessary to permit full distention of the reticular meshes.

In the dog, the tail of the spleen receives its blood supply from small vessels which are also distributed to the adjacent stomach wall. These vessels must be severed in order to remove the spleen, but they must be ligated subsequently in order that the fixative injected into the main vein will not leak out. For cannulating the lienal vein it was found convenient to use no. 20 or 21 syringe needles. It has been possible to attain pressure sufficient to distend the dog spleen maximally only by the use of a syringe. Infusion bottles at convenient levels were tried, but pressures from columns up to 185 cm of 10% formalin failed to distend the organs. Insufficient injection pressure most probably accounts for MacNeal's failure to obtain these results when he attempted to distend human spleens removed at autopsy. The needle must be tied in the vein with a strong ligature, for the spleen offers great resistance to the incoming fluid, and the internal pressure forces the fluid out from around the needle unless the ligature is tight.

The great elasticity of the organ becomes apparent during the injection, for immediately after the barrel of the syringe is removed for a refill the organ shrinks and fixing solution spurts from the needle unless its escape is prevented. Maximal distention is reached when the pressure within the spleen is so great that the fluid escaping between refills is equivalent to that injected. The procedure offers difficulty, since the elasticity of the spleen makes it necessary to pump the fixative into the organ at intervals after maximal distention has already been accomplished. This was not done in the case of the first spleen which was was permitted to contract after distention, and the subsequent contraction of the organ made it almost impossible to follow the crumpled capillaries in 10-μ sections.

After maximal distention is attained, with sufficient fixation to prevent contraction, the vein is ligated and the entire spleen placed in fixing solution. After an hour or so, when the tissue has hardened, a small piece is excised. The excised tissue is left in the fixative for several hours to complete fixation, then dehydrated, embedded, sectioned and stained in the usual manner. In such preparations, providing that compression of the tissue with forceps and other sources of distortion are avoided, one is able to view a vascular architecture that has not been generally recognized in the spleen. It is best to overstain with the cytoplasmic stain in order to accentuate the processes of the cells.

OBSERVATIONS

A characteristic observed grossly in splenic tissue fixed in maximal distention is its sponginess. With pressure, fluid is expressed in quantity, and if the tissue is then dropped back into fluid it gradually regains its former shape; fluid is drawn back into the tissue as its constituents, free from contracting forces, resume the state in which they were fixed. This reaction is not evident in tissue fixed by routine methods for histologic study.

Another characteristic observed in tissue treated in this fashion is its enhanced ability to clear, resulting from dispersion of the elements. Tissue prepared by the above method can be embedded and cut with an ease far exceeding that experienced with blocks of splenic tissue prepared by routine methods. The tissue behaves as lung tissue when it is sectioned from paraffin blocks.

When sections of 10 μ are stained with hematoxylin and eosin, one observes another unusual property of splenic tissue fixed in this manner. Whereas with the same stains, sections from tissue fixed by routine methods give a rich red appearance, since red cells are numerous, sections of this tissue stain a pale pink. The number of tissue cells per unit volume of tissue is markedly reduced, and erythrocytes are almost completely eliminated. With a reduction in the number of cells per unit volume of tissue, it is easy to view in microscopic sections the essential splenic constituents unobstructed by blood cells. Furthermore, because of the internal tension each cell and fiber assumes the form and position that are fairly comparable to conditions within the spleen distended *in vivo.*

Blocks of routinely fixed splenic tissue often show protrusion of nodules of white pulp from the cut surface, an indication of marked compression. The question arises as to how an extensive system of capillaries becomes so completely concealed in sections of routinely fixed tissue. The explanation lies in the marked compression of the splenic reticulum which surrounds the capillaries. Any attempt to discern entwined and collapsed venous capillaries within the compressed reticular meshes is futile, regardless of whether thin or thick sections are used.

The term "venous capillary" has led to some confusion since in the past it has been used synonymously with venous sinusoid. In this account the term venous capillary is applied to the connecting link between the arterial capillary, as described by Robinson, and the true sinus. Sinus is the term used to denote the striated channels previously described in detail by Mollier ('10–'11). Of the two types of venous capillaries described here in the dog spleen the type with the striated wall, structurally resembling a sinus, may be considered by some authors as a small sinus and not a venous capillary. This structure is probably identical with the ''narrow sinus'' of Björkman. However, such features cannot be seen in maximally contracted tissue in which the uncollapsed sinuses are clearly evident. It is felt, therefore, that this structure (fig. 5) should be considered a capillary belonging to the venous system of blood channels. The other type of capillary differs in that no striations have been seen in the wall, which appears to collapse and fold back against the sinus and become obscured in the compressed reticular meshes.

Because of the condensation and compression in the surrounding cellular elements, mostly reticular cells, the venous capillaries were barely discernible in the spleen which was allowed to contract after distention. This spleen exhibited the reticular meshes in a partially contracted state essentially without the interference of fibrin and red cells. The capillaries were kinked and entwined in the reticular meshes. After tracing a distorted capillary through a distance of several sections, the same sections had to be retraced in order to follow the capillary to its termination in a sinus. Many capillaries could not be followed in successive sections because of the obscuring reticular meshes. The junction of the capillary with the sinus formed an acute angle much less than the 45° reported by Weidenreich. These capillaries appeared as flaccid tubes, collapsed and folded back against the more rigid sinus wall. The compact tissue in this spleen made endothelium difficult to study and to photograph.

The spleen that had been fixed in a moderately distended state revealed capillaries distended sufficiently to be followed to a sinus in a single section. Such a partially distended capillary is exemplified in figure 1, as contrasted with a more fully distended one seen in figure 2. A thorough study of the capillaries in sections from this organ showed in many instances a right-angle junction of the capillary with the sinus wall. Some sections showed a capillary apparently connecting two sinuses. However, an adjacent section invariably showed this capillary to be continuous, near its mid-point, with a single capillary of arterial origin. Thus, the capillary apparently connecting the two sinuses was nothing more than the branches of a bifurcating capillary which had been pulled to an angle of 180° by the distending meshes. This relationship was encountered several times in the sections of this spleen, and never was a true capillary without arterial connections seen to join two sinuses. Other capillaries presented crumpled and irregular walls which could be traced to sinuses. No striations, such as those seen in the sinus walls, were observed in the walls of the capillaries studied in this organ. Crumpling of the capillary walls was evidently brought on by shrinkage of the surrounding tissue after the capillary wall had already hardened somewhat.

Some areas of this particular organ showed more marked contraction than others, evident especially in the red pulp. Those portions which were more contracted exhibited small reticular spaces; the pulp exhibited the ''cords'' described by Billroth (1861, 1862). Other areas, more distended, showed wide meshes in the pulp. The small arteries appeared coiled in some areas and straight in others. Some arteries were coiled in one segment, but straight otherwise. The degree of contraction within this organ was seen to vary even within a single highpower field. In one instance two arterial branches could be viewed in one microscopic field. The upper twig, having been subjected to contracting forces, was so irregular that arterioles could not be traced into continuity with the capillary fragments. The lower twig could easily be followed to capillary junctions which in turn were followed into sinuses. The spleen, from these observations, appears to have an exceptionally elastic character even down to its minutest structures.

The spleen that had been fixed in maximal distention revealed the capillary system in clear detail. Distention was the same in all portions of the organ studied. The capillaries were distended and their lumina clearly visible. Every section revealed numerous capillary connections with sinuses. Many of these capillaries were cut in longitudinal section. Where the capillaries were studied in cross-section their arterial extremities were found to be composed of a single layer of endothelial cells similar in structure to those in the vessels passing through the ellipsoid sheaths with which they were continuous. The venous extremities of the capillaries had a much larger lumen and a thinner wall. In instances where the junction of arterial and venous components was observed in cross section the lumen was small and the wall thin. Striations were seen in some of the capillaries (fig. 5) while other capillaries (fig. 7) lacked discernible striations. Longitudinal sections of the junction of the arterial and venous components of the capillaries showed the venous component to be funnel shaped with the narrow lumen proximal and the distal end blending with the reticular meshes (figs. 1 and 2). Closer examination of the distal ends of these venous capillaries always revealed their junction with a sinus in an adjacent section.

Central arteries beginning at their exit from a Malpighian body were followed in all their branches. After leaving the white pulp the artery and its several branches appeared as fairly straight tubes (fig. 7). Within the red pulp all arterial capillaries could be traced to sinuses via venous capillaries. A few arterioles terminating at the border of the white and red pulp appeared to terminate in the reticular tissue alone as no nearby sinuses or capillaries could be seen. These instances were in the minority, however; the vast majority of arterial capillaries were followed readily to sinuses.

Measurements were made on capillaries cut in longitudinal section. The arterial component, beginning at its exit from the ellipsoid sheath, varied between 50 and 80 μ in length. The venous component in some cases was over twice this length. Robinson's figure of 0.1 mm for the arterial capillary evidently included some of the venous component. The ampullae in the dog spleen which Snook has illustrated may be a portion of the venous capillary also.

DISCUSSION

When the spleen contracts, the reticular meshes are compressed into "cords of Billroth." Cords in this sense are not seen in distended tissue; the spaces between the sinuses in such tissue are filled by wide reticular meshes (figs. 2–10). The venous capillaries are hidden in the compressed reticular meshes when splenic tissue contracts and the arterioles appear as free vascular terminals. This is a mechanism exactly opposite to the "open circulation" concept that a tortuous reticular (open) passage may become a straight (closed) passage when the organ contracts. The fact that the sinuses do not collapse in such tissue indicates that they are comparatively stable and resistant to compression.

The venous drainage of the dog's spleen begins with the venous capillary at its junction with the arterial capillary and not at the finger-like projections of the sinusoids which Robinson described in the cat spleen. As to the role played by the venous capillary during contraction and distention of the spleen little can be stated here. The contracted and moderately distended spleens showed variation in the degree of contraction from one part of the organ to another. One might consider that while one portion of the organ is contracting *in vivo* another is expanding. The results of Roy's experiments, however, do not support this concept. His graphs show large waves indicating that the entire organ fills and empties rhythmically; superimposed on the large waves are smaller waves of questionable origin. The filling and emptying of lobular units in groups aside from the filling and emptying of the whole organ may account for the secondary waves of Roy's graphs.

If the preceding is true, then an individual unit probably fills when the arterial capillary dilates and directs the blood stream through the venous capillary into the sinus. It appears from these observations, in confirmation of investigations of the spleen *in vivo,* that the normal pathway of blood cells is through the described capillaries and sinuses and not into the reticular spaces as proposed by those who have favored the ''open circulation'' hypothesis. These observations indicate that the reticular spaces are occupied ordinarily by plasma. Rhythmic contractions of the spleen would force the plasma back into the sinuses. The red cells within the sinuses would then be driven along the sinus channels to the veins. The angle which a venous capillary is found to make with a sinus indicates the amount of contraction at that particular site. This may suggest that as the venous capillaries collapse and fold back during contraction, the retrograde passage of cells is prevented. The infarction resulting when the nerves are cut (Mall) may be due to cessation of the rhythmic contractions and hence loss of the propelling forces which drive the cells along. The exact role played by the venous capillary in these mechanisms requires further study.

SUMMARY AND CONCLUSIONS

A method whereby the dog spleen may be distended for demonstration of its capillaries in routine histologic sections is presented in some detail. The method consists of injecting the organ to the limits of its capacity with formalin or other fixative. The capillaries are extended and readily visible within the dispersed pulp. From attempts to produce distention of the spleen in this study, it is felt that the failure of previous perfusion and injection experiments lay in underestimation of the degree of histologic compression brought about by contraction of the splenic tissue. The following conclusions have been reached:

1. A capillary system connecting arterioles to the sinuses exists in the spleen, thus establishing a ''closed'' blood vascular system.
2. Each capillary is composed of two continuous segments, arterial and venous.

3. The capillaries vary in length. Some have short venous components with a wall structurally resembling that of the sinus. Other capillaries present a longer venous component having a flaccid wall.

4. The capillaries, particularly the venous components, fold and collapse when the spleen contracts, and they extend and open when the spleen is distended.

5. The greatest degree of compression occurs in the reticulum between the sinuses when the spleen contracts. The angle which the venous capillary makes with the sinus wall is a measure of the amount of contraction in that particular portion of the tissue.

6. In moderately distended tissue the venous capillary wall can be sharply demarcated from the sinus wall. The fact that the sinuses remain uncollapsed in the contracted tissue indicates that the sinus wall is more resistant than the capillary wall to compression forces possibly due to a stronger architecture of the sinus wall.

7. In histologic sections of maximally contracted tissue, the capillaries are obscured beyond recognition because they are collapsed, entwined and compressed within reticular meshes.

LITERATURE CITED

BARCROFT, J., AND J. G. STEPHENS 1927 Observations on the size of the spleen. J. Physiol., *64:* 1–22.

BILLROTH, T. 1861 Zur normalen und pathologischen Anatomie der menschlichen Milz. Arch. f. path. Anat., *20:* 409–425.

——— 1862 Zur normalen und pathologischen Anatomie der menschlichen Milz. Arch. f. path. Anat., *23:* 457–487.

BJÖRKMAN, S. E. 1947 The splenic circulation with special reference to the function of the spleen sinus wall. Act. med. scandinav., *191:* 1–89.

HELLY, K. 1903 Die Blutbahnen der Milz und deren funktionelle Bedeutung. Arch. f. mikr. Anat., *61:* 245–273.

HERRLINGER, R. 1949 Neue funktionell-histologische Untersuchungen an der menschlichen Milz. Zeitschr. f. Anat. u. Entwick., *4:* 340–465.

KNISELY, M. H. 1934 Microscopic observations on circulatory systems of living transilluminated mammalian spleens and parturient uteri. Proc. Soc. Exp. Biol. and Med., *32:* 212–215.

KNISELY, M. H. 1936 A method of illuminating living structures for microscopic study. Anat. Rec., *64:* 499–523.

MACKENZIE, D. W., A. D. WHIPPLE AND M. P. WINTERSTEINER 1941 Studies on the microscopic anatomy and physiology of living transilluminated mammalian spleens. Am. J. Anat., *68:* 397–453.

MACNEAL, W. J. 1926 The open circulation of the spleen pulp. Proc. Am. Assn. Path. and Bact., Am. J. Path., *2:* 473–474.

MACNEAL, W. J., S. OTANI AND M. B. PATTERSON 1927 The finer vascular channels of the spleen. Am. J. Path., *3:* 111–119.

MALL, F. P. 1900 The architecture and blood vessels of the dog's spleen. Zeitschr. f. Morph. u. Anthrop., *2:* 30–34.

———— 1902–03 On the circulation through the pulp of the dog's spleen. Am. J. Anat., *2:* 316–325.

MCNEE, J. 1931 The spleen: its structure, functions and diseases. Lettsomian Lectures, *1:* 951–1009.

MOLLIER, S. 1910–11 Ueber den Bau der Kapillaren der Milzvene (Milzsinus). Arch. f. mikr. Anat., *76:* 608–656.

PECK, H. M., AND N. L. HOERR 1951a The intermediary circulation in the red pulp of the mouse spleen. Anat. Rec., *109:* 447–478.

———— 1951b The effect of environmental temperature changes on the circulation of the mouse spleen. Anat. Rec., *109:* 479–494.

ROY, C. S. 1880–82 The physiology and pathology of the spleen. J. Physiol., *3:* 208–228.

ROBINSON, W. L. 1926 The vascular mechanism of the spleen. Am. J. Path., *2:* 341–355.

———— 1930 The venous drainage of the cat spleen. Am. J. Path., *6:* 19–26.

SNOOK, T. 1944 The guinea-pig spleen. Studies on the structures and connections of the venous sinuses. Anat. Rec., *89:* 413–421.

———— 1950 A comparative study of the vascular arrangements in mammalian spleens. Am. J. Anat., *87:* 31–77.

THOMA, R. 1924 Der normale Blutstrom und die venöse Stauung in der Milz. Virch. Arch. f. path. Anat. u. Physiol., *249:* 109–111.

WEIDENREICH, F. 1901 Das Gefasssystem der Milz des Menschen. Arch. f. mikr. Anat., *58:* 313–315.

PLATES

ABBREVIATIONS

A, artery
AC, arterial capillary
E, ellipsoid
S, sinus
Tr, trabecula
VC, venous capillary

All sections were $10\,\mu$ in thickness and stained with hematoxylin and eosin.

PLATE 1

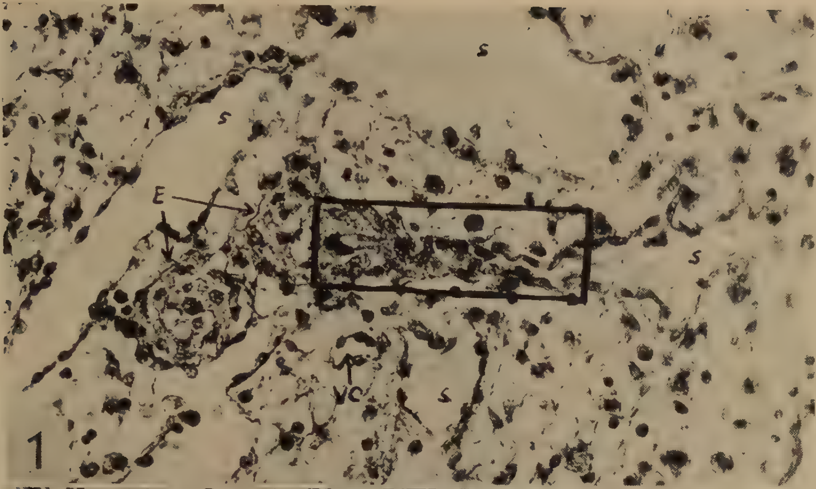
EXPLANATION OF FIGURES

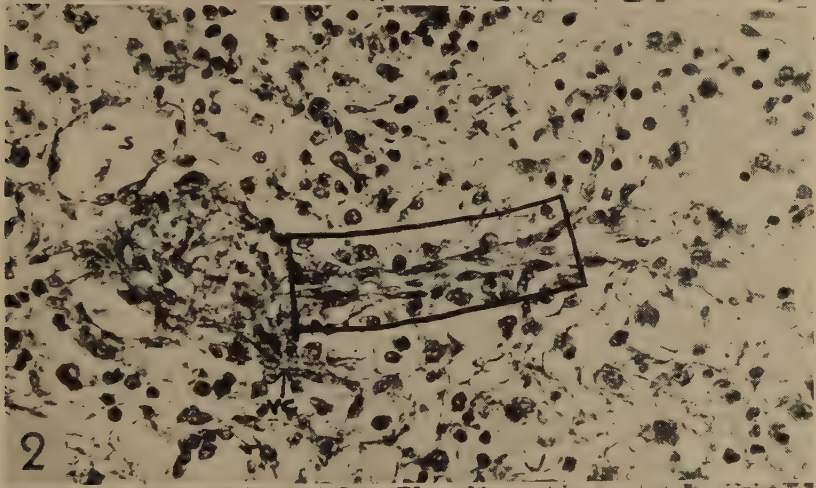
1 Longitudinal section of a partially compressed capillary from a moderately distended spleen. Note the venous component as compared with the relatively straight arterial component. The sheath has been sectioned obliquely. Note the cross section of the venous capillary as compared to the cross section of the arterial capillary within the ellipsoid sheath on the left. × 361.

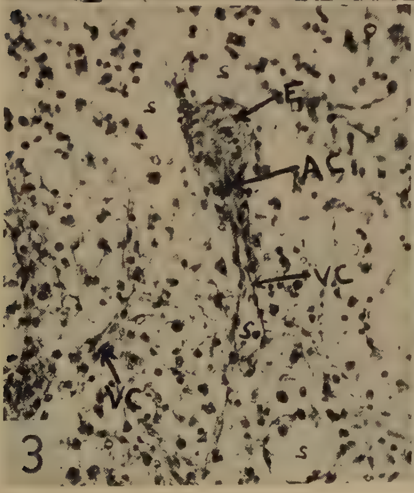
2 Longitudinal section of a completely distended capillary from a maximally distended spleen. Compare with the corresponding structure in figure 1. Note how few sinuses can be seen, an indication of more distention. The capillary appears to blend with the reticulum here, but the adjoining section in the series revealed sinus connections with the venous component. × 361.

3 Structure similar to the one in figure 2, showing in addition the connection with a sinus. × 214.

4 Bifurcating venous capillary. Note its relation with the trabecula. × 214.


S
S
E
S
VC
S
1


S
VC
2


S
S
E
AC
VC
S
VC
S
3

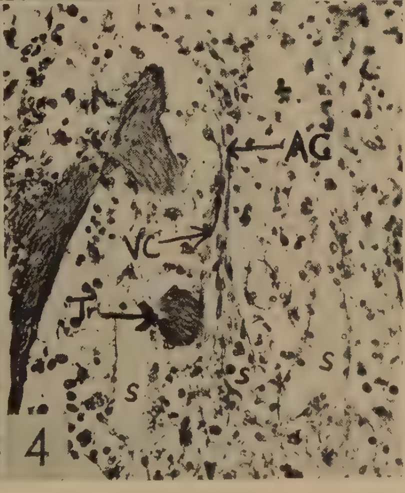

AG
VC
Jr
S
S
S
4

PLATE 2

EXPLANATION OF FIGURES

The following sections were from a maximally distended spleen.

5 Striated capillaries (top arrow). The wall of this type of capillary resembles structurally the sinus wall. × 214.

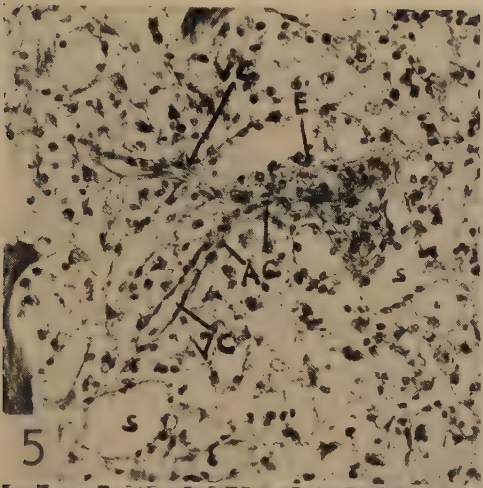
6 Longitudinal section of a venous capillary showing its entrance into a sinus. Note how the reticulum surrounds the capillary as it passes into the sinus. Had the section missed the sinus this capillary would have appeared exactly as the capillary in figure 2, passing into reticular spaces. × 214.

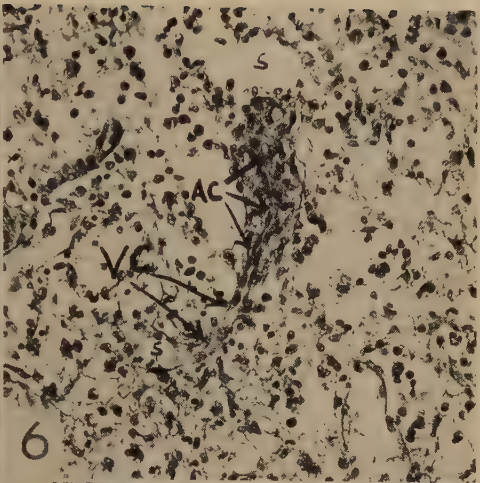
7 This section shows an exceptionally long venous capillary joining two sinuses. This type of capillary was seen frequently in the spleen that was only moderately distended. Note the uniform lumen of the section of the artery in this section. In the contracted and moderately distended spleens studied this structure often showed coiling. × 214.

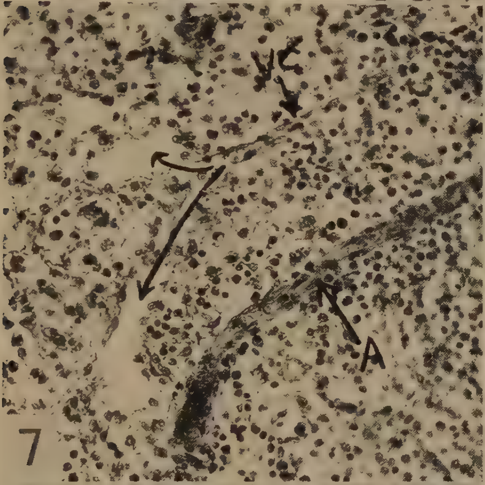
8 Longitudinal section of arterial capillary and its venous component. The venous capillary bifurcated and only one branch is shown in this section. × 214.

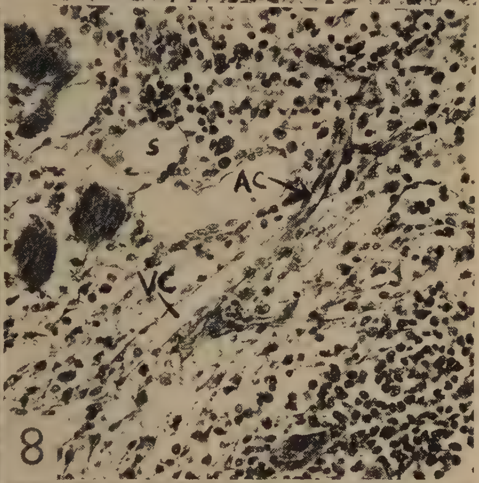
9 Longitudinal section of an entire capillary making its exit from the sheath and bifurcating. Isolated cuts through venous capillaries are seen on the left. × 214.

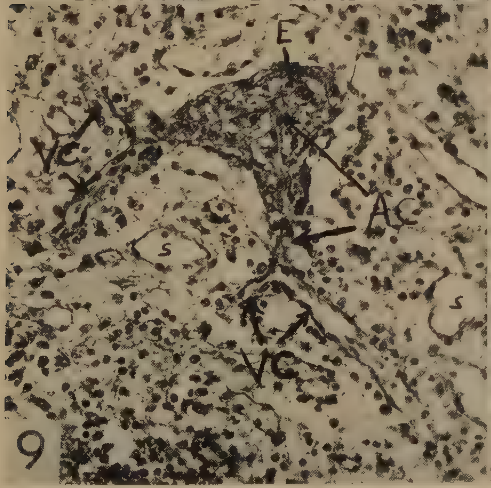
10 Longitudinal and cross sections of ellipsoids revealing the structure of the capillary walls. × 214.

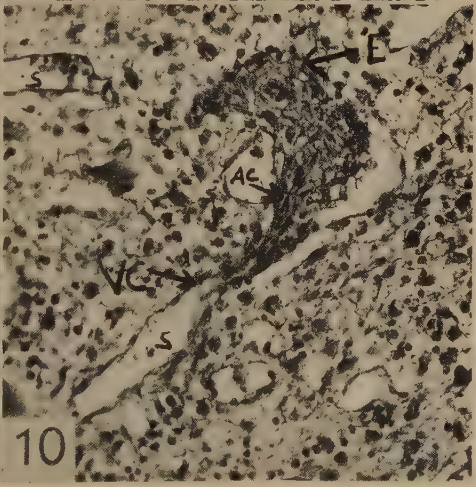

5


6


7


8


9


10

THE GROWTH PATTERN OF THE CRANIAL VAULT IN THE ALBINO RAT AS MEASURED BY VITAL STAINING WITH ALIZARINE RED "S"

MAURY MASSLER[1] AND ISAAC SCHOUR
University of Illinois, College of Dentistry, 808 South Wood Street,
Chicago 12, Illinois

FOUR FIGURES

INTRODUCTION

The purpose of this investigation was to study the growth pattern of the cranial vault in the albino rat. An attempt was made to designate: (a) the sites of growth, (b) the mode of bony growth at these sites, (c) the chronology and (d) the rates of growth at the different sites.

This program was made possible by the fact that alizarine red "S," when injected as a vital dye, selectively stains actively growing bone (and dentin) (Schour, '36).

REVIEW OF LITERATURE

The growth of the cranial vault has been often deduced by comparison of young and adult crania (Hatai, '07; Krogman, '30). Its growth has been investigated only infrequently by direct experimental methods (Troitzky, '32).

Brash ('34) used the indirect method of vital staining by means of madder feeding in the pig. He explained the growth of the cranial vault by a simple mechanism of external (ectocranial) deposition and internal (endocranial) resorption. He ascribed to the sutures only the secondary role of adjustment.

---

[1] Submitted in partial fulfillment of the requirements for the Degree of Master of Science in the Graduate School of the University of Illinois.

METHODS AND MATERIALS

Vital injections of alizarine red "S" stain the bone, dentin and cementum growing and calcifying at the time of the injection. It is, therefore, a vital stain for all the calcifying tissues except enamel (Schour, '36). The direct method of alizarination consists of giving multiple injections of the dye at selected intervals. After sacrifice, the gross specimens or ground histologic sections show the injection effects as separate lines which can easily be measured. The indirect method can be used only in the young, rapidly growing animal. It consists of giving a single injection which stains all the bone present at that time. This is so since all the bones are actively growing. A sufficient interval is then

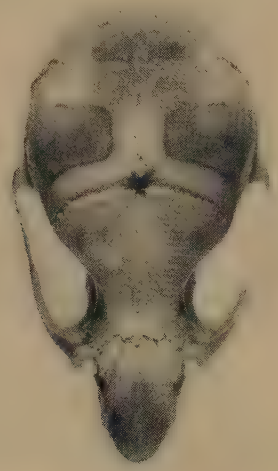


Fig. 1 Cranium of albino rat virtually injected with alizarine red 'S' at one day of age and sacrificed at 20 days of age. Dark areas, red-stained bone growing at the time of the injection. Light areas, bone formed between one and 20 days of age.

allowed to elapse before the animal is sacrificed. The new bone formed after the injection appears white (fig. 1).

The indirect method of alizarination was used in this study. One hundred and thirteen albino rats were each given a single intraperitoneal injection of a 2% solution of alizarine red "S" at various ages between birth and 310 days and were sacrificed three to 90 days later (table 1). The dosage ranged from 75 to 100 mg per kilogram of body weight. The animals were a well-standardized, modified Wistar strain bred by the Department of Physiology of the University of Illinois.

The analyses were made upon the gross macerated wet specimens. The bones growing at the time of the injection were stained red by the alizarine. The new bone growing after this time was uncolored (white) (fig. 1). In this manner the sites of growth as well as the chronology and rate of growth at these sites were made apparent. Measurements of the amount of new white bone were made from the red edge of one bone to the red edge of the next at right angles to the growing surfaces. The measurements were made under a dissecting microscope with a caliper and were read against a stage slide divided into 10ths of a millimeter (table 1).

OBSERVATIONS

Periods of generalized and localized growth

Vital injections of alizarine red "S" in rats *under 60 days of age resulted in a red staining of all the bones present at that time.* Microscopic analysis (unstained ground sections) showed that this generalized staining in the young animal was due to the fact that bone growth was progressing upon all bony surfaces — periosteal, endosteal, intersutural and endochondral. *Injections of the dye in animals over 70 days of age resulted in the red staining of only certain localized areas,* indicating that after the 70th day of age, bony growth was confined to localized sites.

TABLE 1

Amount of postnatal bone growth (in mm) at various growth sites at different ages

GROUP	NO. ANIMALS	AGE AT INJECTION	AGE AT SACRIFICE	INTERVAL	INTERNASAL SUTURE	METOPIC SUTURE	SUPERIOR MARGIN OF FORAMEN MAGNUM	INTER OCCI- PITA SUTUR
		In days						
I.	8	0	3	3	0.5	*	*	*
		0	10	10	0.5	1.7–3.5	0.6	1.8
II.	6	2	12	10	0.1	1.5–3.0	0.6	2.0
III.	4	5	15	10	0.0	0.5–1.0	*	1.2
IV.	25	10	15	5		0.0	*	0.5
		10	20	10			0.2	0.8
		10	30	20			0.7	1.2
		10	40	30			1.2	1.5
		10	70	60			1.3	1.5
		10	100	90			0.0	0.0
V.	16	20	40	20				
		20	50	30				
		20	100	80				
VI.	7	30	60	30				
VII.	10	41	71	30				
		41	101	60				
		41	131	90				
VIII.	8	60	90	30				
IX.	9	70	100	30	Growth ceases at 3 days of age	Growth ceases at 10 days of age	Growth ceases at circa 20 days of age	Growt ceases at circa 20 day of ag
		90	120	30				
		100	130	30				
X.	20	120	150	30				
		131	221	90				
		220	250	30				
		250	310	60				

* Growth was so slow that the amount of new bone formation could not be measured grossly. New bon formation was, however, discernible.

TABLE 1 — *continued*

Amount of postnatal bone growth (in mm) at various growth sites at different ages

AGITTAL SUTURE	CORONAL SUTURE	POSTERIOR LAMBDOIDAL SUTURE	ANTERIOR LAMBDOIDAL SUTURE	ANTERIOR MARGIN OF NASAL BONE	FRONTO-NASAL SUTURE	CIRCUM-TEMPORAL SUTURE	TEMPORAL CREST
*	*	*	*	*	*	*	..
2.5	2.6	1.5	2.6	1.7	5.0	*	..
2.5	2.7	1.8	2.8	1.3	4.8	2.0	..
1.8	1.5	1.0	1.5	0.8	3.5	1.0	..
0.3	0.7	0.4	0.7	0.6	1.5	0.3	..
0.6	1.0	0.6	1.0	0.9	2.5	0.8	..
0.6	1.2	0.8	0.9	1.2	4.6	0.8	..
0.8	1.6	1.5	1.3	1.8	6.5	*	..
1.3	1.8	1.7	1.8	2.0	9.0	*	..
1.2	2.3	1.7	2.0	2.4	10.0	*	..
0.0	0.7	*	0.7	1.0	2.7	*	..
	0.8	*	0.8	1.3	5.0	*	..
	1.2	*	1.2	1.8	7.0	*	..
	0.0	0.0	0.6	1.1	2.3	*	0.6
			0.0	*	1.3	*	0.5
				1.0	1.6	0.2	0.9
				1.2	2.0	*	1.1
Growth ceases at 0 days of age	Growth ceases at 30 days of age	Growth ceases at 25–30 days of age	Growth ceases at 30–40 days of age	*	*	*	*
				Growth ceases at circa 70 days of age	*	*	*
					*	*	*
					*	*	*
					*	*	*
					*	*	*
					*	*	*
					*	*	*

---

* Growth was so slow that the amount of new bone formation could not be measured grossly. New bone rmation was, however, discernible.

Sites of growth

Increase in cranial size

Intersutural growth. This study revealed the sutures to be the most prolific sites of postnatal growth in the cranium. Intersutural bone growth was very rapid and quite evident from birth to 30 days of age. The rate of growth decreased markedly with age (age gradient), so that after the 30th day of life growth was confined to only certain of the sutures.

Increase in skull width. Increase in the width of the skull was accomplished by growth at the sagittal complex of sutures. The sagittal complex consists of three segments: (a) the internasal suture between the two nasal bones of the snout, the major site of increase in nasal width; (b) the metopic or interfrontal suture between the two frontal bones which contributed to the increase in bifrontal width; and (c) the sagittal suture proper between the parietal bones. The sagittal suture was the site mainly responsible for the increase in the maximal width of the cranial vault (figs. 1 and 2).

A part of the increase in snout width was contributed by the lateral growth of the frontal process of the maxillae. This dimension is not considered in this report and the growth of the binasal width at the internasal suture alone was studied.

Increase in skull length. Cranial vault. Increase in the length of the cranial vault was accomplished primarily by intersutural growth at the coronal and the anterior and posterior lambdoidal sutures. The anterior lambdoidal suture is the lambdoidal suture proper, while the posterior lambdoidal suture is an accessory suture formed by the presence of the interparietal bone between the parietals and the occipital bones (figs. 1 and 2).

Snout. The frontonasal suture and the anterior margin of the nasal bones contribute to the increase in snout length. The frontonasal suture was the most prolific site of intersutural growth in the entire skull, a condition to be expected of a long-snouted animal (tables 1 and 3 and fig. 2).

Increase in vault height. Increase in cranial height was accomplished by growth at (a) the superior margin of foramen magnum (which, unlike man, opens posteriorly);

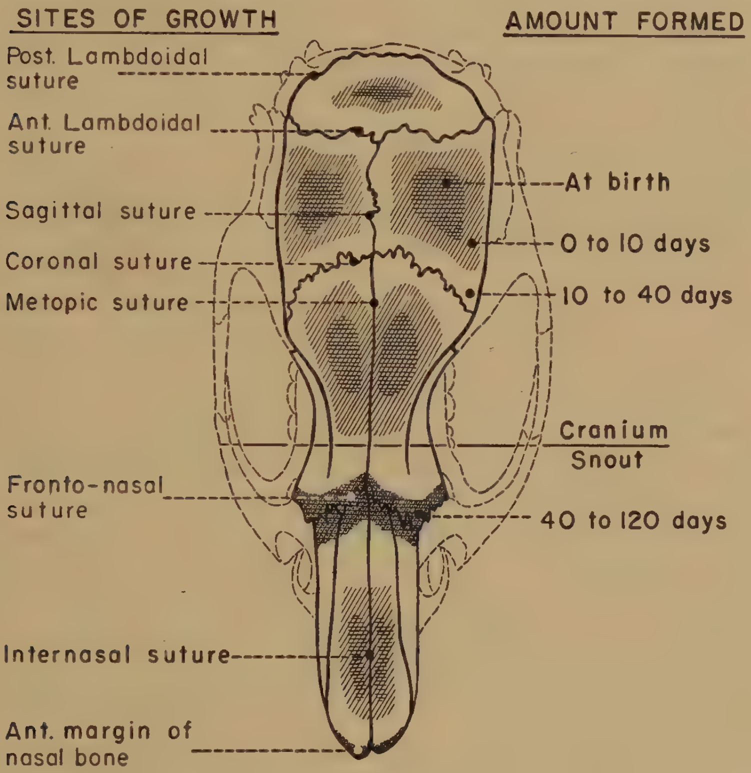


Fig. 2 Diagram indicating the sites and amount of growth in the cranial vault of the albino rat.

(b) the interoccipital suture lying between the supra and superior portions of the occipital bone; and (c) the circumtemporal suture. The interoccipital suture in man begins to close at three months in utero and is present only as a cleft or fissure at birth. In the albino rat, this suture was a site of active bone deposition until about 20 days of age.

In the animal under 30 days of age, the anterior and posterior lambdoidal sutures joined laterally and met with the interoccipital and circumtemporal sutures at the posterior lateral or mastoid frontanelle above the tympanic bulla. The interoccipital suture was therefore continuous anteriorly with the circumtemporal suture and posteriorly with the superior margin of foramen magnum. These three areas, the free margin of the occipital bone forming the superior margin of foramen magnum, the interoccipital suture and the circumtemporal suture thus formed a continuous site of growth at the side and back of the cranial vault. This complex ran parallel to the dorsal aspect of the cranial vault and growth at these sites contributed the major portion of the increase in vault height.

Increase in thickness of vault bones

Growth on ectocranial and endocranial surfaces. Increase in the thickness of the vault bones was accomplished by the deposition of bone upon the ecto- and endocranial surfaces. The red staining was readily seen upon both the ecto- and endocranial surfaces in all animals *less than 70 days of age.* The red staining was especially vivid upon the endocranial surface. Sections cut through the vault and examined under low power showed a vivid staining of the endocranial surface of the inner table and of the diploe. There was only mild staining on the ectocranial surface of the outer table. These findings do not agree with those of Brash ('34) who found, in the pig, new bone growth on the ectocranial surface with resorptions of the endocranial surface.

After the 70th day of age, the red staining was confined to only certain areas on the ectocranial surfaces: the area over the frontal sinuses; the frontonasal suture; the temoral crest and circumtemporal suture complex; the nuchal area of muscular attachments and the zygomatic arch. These sites of growth persisted up to (and probably beyond) 300 days of age.

Rates and chronology of growth

The absolute rates of growth at the various sites of growth studied are tabulated in table 1. In all cases the rate of growth was most rapid at birth and decreased markedly with age (age gradient).

The *relative* rates of growth at the different sutures are perhaps of greater interest than are the absolute rates since these are responsible for the changes in proportions that characterize development.

Sagittal complex (increase in cranial width). From table 1 it can be seen that the sagittal complex of sutures grew very slowly and only for a short period of time. Growth at the internasal and the interfrontal (or metopic) portions was completed by the 3rd and 10th days of age, respectively (tables 1 and 2). The sagittal suture proper grew a little more rapidly and for a longer time, growth being completed at this site by the 20th day of age (tables 1 and 2).

Since the sagittal complex of sutures contributed to skull width, it can be seen that nasal width was completed by the third day and minimal bifrontal width was virtually completed by the 10th day, while the maximal cranial width (or bitemporal-crest distance) was attained by the 20th day.

Coronal and lambdoidal sutures (increase in cranial length). The sutures which contributed to the increase in cranial length grew more rapidly and for a longer period of time than did the sutures which contributed to the growth in width. The coronal and lambdoidal sutures not only grew at a more rapid rate than did the sagittal sutures, but growth at these sites persisted for a longer time — until the 40th day of age. Maximal vault width was virtually completed by the 20th day of life while the increase in vault length was not completed until the 40th day. In addition, growth in length was accomplished at three sites (coronal, anterior and posterior lambdoidal sutures), while growth in width occurred at only a single site (sagittal sutures). These three factors (greater rate of growth, longer duration, larger number of growth sites) readily explained how the round head of the new-born rat became the elongated tennis racket-shaped head of the adult rat (fig. 3).

Frontonasal suture and anterior margin of the nasal bones (increase in nasal length). Growth at the anterior and posterior (frontonasal) margins of the nasal bones was very rapid and resulted in a marked and rapid increase in the

TABLE 2

Sites and chronology of postnatal growth of bone in the cranial vault and snout of the albino rat

			AGE AT WHICH GROWTH CEASES
I. Increase in width —			
	Sagittal complex of sutures		
		1. Internasal suture	Circa 3 days
		2. Metopic (interfrontal) suture	10 days
		3. Sagittal suture	20 days
II. Increase in length —			
	(A) Cranial vault		
		1. Coronal suture	30 days
		2. Anterior lambdoidal suture	30–40 days
		3. Posterior lambdoidal suture	25–30 days
	(B) Snout		
		1. Anterior margin of nasal bone	Circa 70 days
		2. Frontonasal suture	Active after 300 days
III. Increase in height —			
		1. Superior margin of foramen magnum	Circa 20 days
		2. Interoccipital suture	Circa 20 days
		3. Circumtemporal suture	Active after 300 days
IV. Increase in thickness of vault bones —			
		1. Ectocranial surface (generalized)	60–70 days
		2. Endocranial surface (generalized)	Active after 300 days

length of the nasal bones. Growth at the sutural margin was much more rapid and continued for a longer period of time than at the free anterior margin. This bone lends itself to an interesting and provocative study of the rate and mode of bone growth at a free and sutural margin.

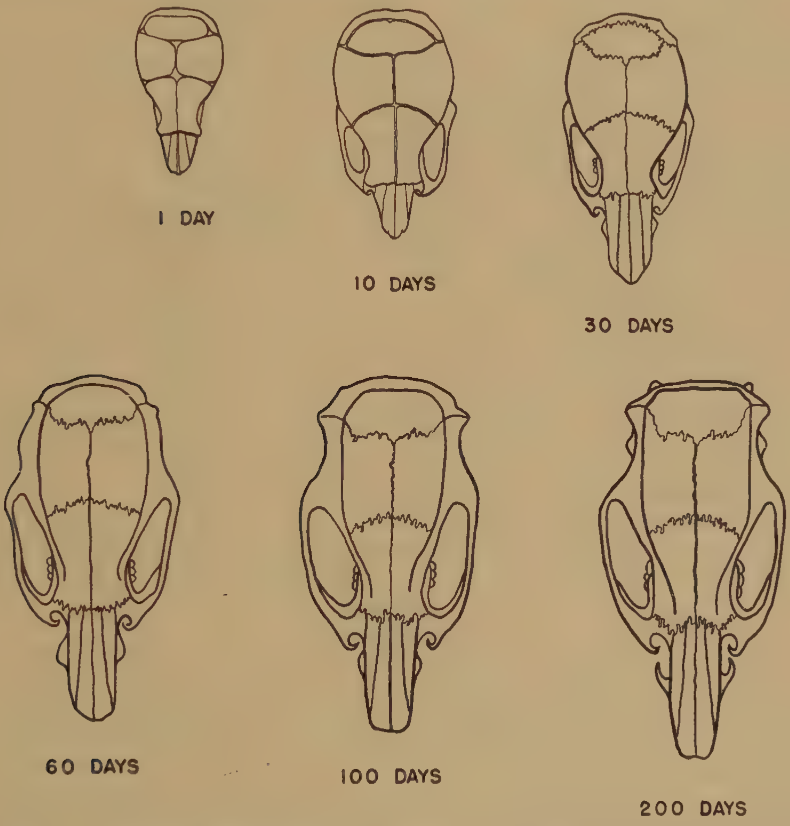


Fig. 3 Drawings illustrating the growth of the cranial vault in the albino rat from one to 200 days.

The frontonasal suture was the site of most rapid growth in this study (table 1). Its growth continued until the 300th day of life (possibly longer), while the free anterior margin of the nasal bones ceased its growth by about the 70th day. The rapid increase in the length of the nasal bones gave to this animal its typical long-snouted appearance, a characteristic which progressed with age. The increase in nasal length occurred more rapidly and earlier in the male than in the female (Hatai, '07).

The snout was quite small in the newborn, while the cranium was relatively large. This relationship is typical of most mammals, including man. In the rat, however, the snout grew at a relatively more rapid rate than did the cranium.

Endo- and ectocranial bone deposition. It was not possible to measure the rate of endo- and ectocranial surface deposition of bone in the rat because of the thinness of the bones and the difficulty in preparing adequate ground sections. This phase of the problem will be analyzed by vital staining of the crania of rhesus monkeys.

Growth potential. It is interesting and perhaps significant to notice the close correlation betwen the rate of growth at a given site and its chronology. Growth tends to persist for a longer period of time at those sites where the initial rate of growth was more rapid. It would appear that different sites enjoy different growth potentialities and that a greater amount of growth energy at a given site is expressed at a faster rate and over a longer period of time.

Sex differences

Differences in the rates of growth at the various sites in the male and female could be distinguished by the 30th day of life but did not become prominent until after puberty (60 days). The female grew at a slower rate than did the male, and the various sites of growth terminated at a slightly earlier age. The female skull was therefore similar to a male skull of an earlier chronologic age level. While the absolute difference between the male and female at a given site was quite small, the *cumulative* effect could be readily seen in the smaller size and relatively immature proportions of the female cranium after 100 days (fig. 4).

Whether the slower rate of growth in the female cranium was the result of a steeper gradient of growth, a smaller growth potential, or both, could not be determined by our methods. This question (whether sex differences in growth stem from differences in growth potential or growth gradients) is fundamental to the understanding of the phenomenon of growth.

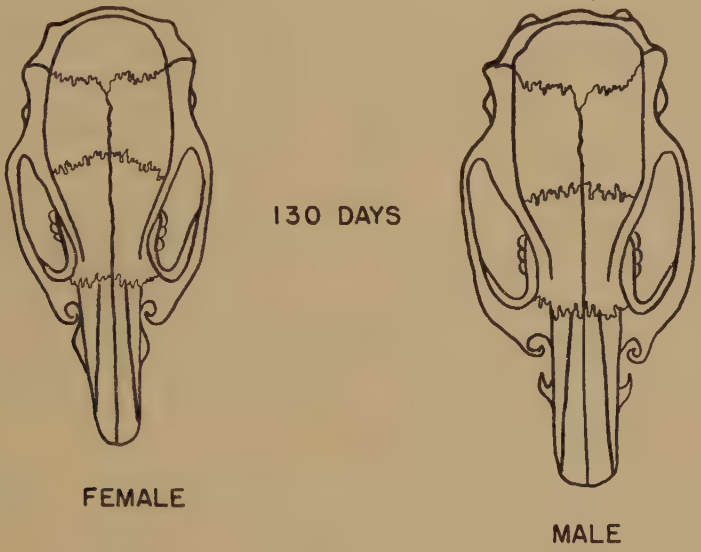


Fig 4 Drawings illustrating differences in the cranial vaults of female and male albino rtas at 130 days of age.

DISCUSSION

The growth of the cranial vault is intimately connected with the growth of the brain and follows the same type of neural growth curve.

Some texts assume that the cranial cavity enlarges by external surface deposition and internal resorptions. A simple mechanism of apposition and resorption such as suggested by Brash ('34) can hardly account for the great velocity of cranial growth during infancy. Similarly, aberrations in cranial development such as occur in hydrocephalus and in premature synostoses cannot be understood or explained by such a simple mechanism. This study emphasizes the primary role of the sutures in cranial development.

Vital staining of bone with alizarine red "S" shows that there are, in general, two periods of skeletal growth:

1. A period of *generalized bony growth* from birth to about 60 days of age, which was characterized by the active deposition of bone upon all bony surfaces. The rate of growth at different sites did vary, however (table 1). This period is characterized by a very rapid increase in the size of the cranial vault with only minor changes in proportions. The latter are due to differences in the rate of growth at the different sites.

2. A period of *localized growth* after 70 days of age characterized by the fact that bone deposition occurs only at certain sites. These sites were, in general, the same that showed the most rapid rate of growth during the previous period. Since growth is confined to localized areas, the predominant characteristic of this period is a marked change in the proportions of the cranial vault with only a relatively slight increase in size (fig. 3).

The rat skull is relatively round at birth (fig. 3). As growth continues, the increase in vault length is more rapid and extends over a longer period of time than does the increase in vault width. By the 30th day of life, therefore, the head becomes markedly elongated. As growth continues from the 30th to the 60th day of life, further elongation occurs (fig. 3).

After the 60th day of life when the period of generalized ectocranial growth is completed, growth is confined to only a few localized areas. The most prominent site of red staining after 60 days is over the frontal sinuses. These areas correspond to the supraorbital ridges of the anthropoids and apparently serve the same purpose — to permit enlargement of the frontal sinuses by resorption from within. In addition, the areas of muscular attachments continue to grow during the period of localized growth. The temporal crest becomes prominent after 60 days of age and continues to grow throughout the normal life-span of the animal. This growth is more prominent and rapid in the male than in the female.

The growth of the snout at the frontonasal suture contributes markedly to the changes in proportions that characterize maturity. From a round-headed, small-snouted animal at birth, the rat becomes a mesocephalic animal, with a medium-sized snout. By the 60th day the snout begins to assume major proportions. It continues to increase in relative size after the 60th day, whereas the cranium remains static. By the 100th day of life, the rat head assumes the proportions and shape of a tennis racket. Growth of the snout in the male is more vigorous and continues for a longer period than in the female.

Modes of growth

Bone growth at the ectocranial and endocranial surfaces (inner and outer tables) occurred by simple, surface deposition of bone, one layer upon the other. This mode of growth produced a compact type of bone composed of circumferential lamellae. The diploe (spongy bone) were formed by concomitant resorptions from within, as were the frontal sinuses.

The intersutural growth of bone was very interesting from many aspects. A given sutural line began (before birth) as the smooth periphery of a bone. As growth continued at the free margin of the bone, it made contact with the periphery of the adjacent bone and thereafter these margins became serrated and interdigitated.

It is well established that under the influence of tension, a given osteogenic layer will deposit a trabecular type of bone instead of simple circumferential lamella. This mode of bone growth is well illustrated in the alveolar process of the erupting tooth. A gomphosis type of attachment similar to that present around the tooth root exists at the actively growing suture. The two ends of the bones are joined together by the peg and hole arrangement of the bony trabeculae and the whole held firmly in place by strands of fibrous connective tissue. The osteogenic layer upon the endo- and ectocranial surfaces is continued between the sutures. This arrangement permits slight mobility at the sutural junction and rapid deposition of bone at the same time.

The rapidly expanding brain encased within the cranial vault exerts a definite internal pressure upon the bones of the vault. This pressure is translated into a tension force upon the intersutural periosteum. The intersutural ostegenic layers under the influence of this tension form a trabecular type of bone instead of the simple circumferential layers of bone seen upon the ecto- and endocranial surfaces. As growth continues at the free margins under such tension, the trabeculations become more and more apparent and gross (fig. 3).

TABLE 3

Correlation between the height of sutural serrations and the chronology of growth at that suture in the albino rat

	AGE AT WHICH GROWTH CEASES	HEIGHT OF SERRATIONS IN ADULT MALES AGE 220 DAYS
1. Internasal suture	Circa 3 days	0.0 mm
2. Metopic suture	10 days	0.0 mm
3. Sagittal suture	20 days	0.0–0.3 mm
4. Interoccipital suture	Circa 20 days	0.0–0.2 mm
5. Posterior lambdoidal suture	25–30 days	0.0–0.5 mm
6. Anterior lambdoidal suture	30–40 days	0.4–0.7 mm
7. Coronal suture	30 days	0.8–1.2 mm
8. Circumtemporal suture	Active at 300 days	(overlapped)
9. Frontonasal suture	Active at 300 days	1.0–2.0 mm

The serrations which characterize the sutures are therefore gross manifestations of this mode of trabecular growth.

This postulate is supported by the work of Troitzky ('32). When complete or partial ablation of a given suture was performed so that there could be no intersutural tension, the free margin of the parietal bone grew as a smooth line. When the bone again made fibrous contact with its neighboring bones and was once more under a tension force, serrations and interdigitations again became manifest.

Examination of the serrations at the different sutures further supports this hypothesis. Sutures which closed early were not serrated, while those at which growth occurred for a longer period of time were highly serrated (table 3). The correlation between the age at which growth ceases at a given suture and the height of the serrations was good (table 3).

SUMMARY

The growth pattern of the cranial vault was investigated by means of vital staining with alizarine red "S" in 113 rats from birth to 300 days of age.

1. There are two periods of growth in the skeletal system of the rat. In the first, there is a rapid deposition of bone upon all bony surfaces. In the second period (after 60 days of age), growth is localized to certain definite sites.

2. The growth of the cranial vault occurs primarily by a rapid deposition of bone at the approximating margins of the bones which comprise the various cranial sutures. The mode of growth (by trabeculation) and the rapid rate are apparently a response to the rapidly expanding encased brain. Sutural serrations are a gross manifestation of the mode of trabecular growth.

3. The sutures are so arranged in relation to one another as to permit each group to contribute to the increase in skull width, skull length and cranial height. The sites concerned with growth in each dimension are tabulated in table 1.

4. Deposition of bone also occurs upon the endo- and ectocranial surfaces to increase the thickness of the vault bone. Concomitant internal resorptions form the diploe and frontal sinuses.

5. The changes in the proportions of the cranial vault and snout are determined by (a) the number of growth sites in a given direction, (b) the rate of bone deposition at a given site and (c) the length of time over which growth at that site is active. Skull length increases more rapidly than skull width because all three factors are greater in the direction of length over width.

The sites of growth and their chronology as well as the rates of growth at each site for the different ages are indicated in table 1.

6. The order in which growth ceases at the various sutures and the relation to the height of the suture serrations are indicated in table 3.

Binasal width is completed by the 3rd day; minimal bifrontal width by the 10th day; maximal cranial width is attained by the 20th day. Increase in vault length is virtually completed by the 40th day, while snout length continues to increase to (and possibly beyond) 300 days.

7. The female cranial vault grows at a slightly slower rate at the various growth sites than does the male, giving to the female skull the appearance of a less mature male skull. It is not known whether this is due to a smaller growth potential or a greater gradient of growth.

REFERENCES

Brash, J. C. 1934 Some problems on the growth and developmental mechanics of bone. Edinburgh Medical J., *41:* N. S. IV.

Greene, E. 1935 Anatomy of the rat. Trans. Am. Phil. Soc., *27,* new series.

Handelsman, M. B., and E. F. Gordon 1930 Growth and bone changes in rats injected with anterior pituitary extract. J. Pharmacol. and Exp. Therap., *38:* 349–362.

Hatai, S. 1907 Studies on the variation and correlation of skull measurements in both sexes of mature albino rats. Am. J. Anat., *7:* 423–442.

Krogman, W. M. 1930 The problem of growth changes in the face and skull as viewed from a comparative study of anthropoids and man. Dental Cosmos, *72:* 624–630.

Schour, I. 1936 Measurements of bone growth by alizarine injections. Proc. Soc. Exp. Biol. and Med., *34:* 140–141.

Schour, I., M. M. Hoffman and B. G. Sarnat 1941 Vital staining of growing bones and teeth with alizarine red "S." J. Den. Res. *20* (5): 411–418.

Schour, I., and M. Massler 1940 Postnatal craniofacial and skeletal development in the albino rat and the Macacus rhesus monkey as demonstrated by vital injections of alizarine red "S." Anat. Rec., *76:* no. 2, suppl. no. 2, Feb. 24, 94.

Todd, T. W., and D. W. Lyons 1924 Endocranial suture closure: I. Adult males of white stock. Am. J. Phys. Anthrop., *7:* 325–384.

———— 1925 Ectocranial suture closure: II. Adult males of white stock. Ibid., *8:* 23–46.

Troitzky, Von Wl. 1932 Zur Frage der Formbildung ded Schädeldaches (Experimentelle Unterschung der Schädeldachnähte und der damit verbundenen Erscheinungen). Zeits. f. Morphol. and Anthropol., *30:* 504–530.

A PHYSIOLOGICAL AND ANATOMICAL STUDY OF THE CLOSURE OF THE DUCTUS ARTERIOSUS IN THE DOG[1]

NEWTON B. EVERETT AND ROBERT J. JOHNSON
Department of Anatomy, University of Washington
School of Medicine, Seattle, Washington

FIVE FIGURES

INTRODUCTION

The recent application of isotopic tracers to the study of fetal circulation has given new evidence concerning the degree to which the two caval blood streams are mixed in the heart of fetal dogs and guinea pigs (Everett and Johnson, '50). In view of this, it has seemed justifiable to apply these improved and more sensitive methods to the important problems of the changes in circulation following birth. One of these problems is the time of closure of the ductus arteriosus, which during development diverts part of the blood from the pulmonary trunk into the dorsal aorta.

The cineradiographic studies of fetal circulation in the lamb (Barclay et al., '41) support the conclusion of Gérard ('00), that the ductus arteriosus closes functionally very soon following birth and that anatomical obliteration embraces a considerably longer period. Similarly, Hamilton, et al. ('37) concluded that in the dog and rabbit there is an early functional closure of the ductus arteriosus. In further support of an early functional closure is the work of Kennedy and Clark ('41), who found that in the guinea pig the ductus arteriosus was always closed when examined between 4 and 10 minutes following the onset of effective respiration.

---

[1] This investigation was supported in part by a research grant from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

Patten ('46), however, has indicated the need for caution in evaluating the evidence supporting the concept of immediate functional closure. He has pointed out that the initial tendency for the circular smooth muscle to contract does not necessarily imply a sustained contraction that would prevent the flow of blood during the weeks occupied by the anatomical closure.

The present report concerns a correlated physiological and anatomical study of the ductus arteriosus from the late fetal period to the time that anatomical obliteration is complete.

MATERIAL AND METHODS

Fetal and newborn dogs which ranged in age from one week prepartum to three weeks postpartum were used in this study. The fetal specimens were from time-mated dogs and the postpartum specimens were ones whose time of delivery was accurately known. The fetuses were delivered by caesarean section under nembutal anesthesia. Their placental circulation was maintained intact and breathing was prevented by keeping the amnion with entrapped amniotic fluid over the head. A combination of ether and nembutal anesthesia was used for the postpartum specimens. Electrosurgery was employed to remove the superior part of the left thoracic wall, thus exposing the vessels pertinent to the study with a negligible loss of blood. The mediastinum was kept intact until the moment preceding the injection and sampling procedure; thus the right lung continued to function except for the last few seconds of the operation. The left lung was reflected ventralward and medialward and a sampling needle was inserted into the left pulmonary artery and another into the dorasl aorta just distal to the ductus arteriosus. Two-tenths of 1 cm^3 of physiological saline labeled with radioactive phosphorus (P^{32})[2] was introduced into the right ventricle and .1 cm^3 of blood was withdrawn into each of the sample chambers. The injection and sampling procedure was essentially the same as that described previously (Everett and Johnson, '50). The only samples used were those in which the blood flowed evenly and at an equal rate into both tubes. These were collected within 5 seconds from the time the needle was first introduced into the right ventricle. A series of control experiments revealed that this interval was less by one second or more than the time required for the tagged blood to make the pulmonary circuit and to reach the dorsal aorta at the point of its juncture with the ductus arteriosus. These control experiments were made on a series of fetal and newborn animals after ligating the ductus arteriosus.

---

[2] The radioactive phosphorus used in this investigation was supplied by Clinton Laboratories on allocation from the Isotopes Division, U. S. Atomic Energy Commission.

The blood samples were spread evenly on cupped planchets and dried with an infra-red lamp. Radioactive measurements were made by conventional counting methods and the ratio of activity between the pulmonary and aortic samples was determined for each specimen.

For each age group represented in this study, at least one ductus arteriosus with the adjacent portions of the pulmonary artery and dorsal aorta was excised and prepared for histological examination.

OBSERVATIONS

The ratios in terms of radioactivity between the blood samples from the dorsal aorta and the pulmonary artery of late fetal specimens ranged from 1:1 to 2:1 (table 1). Three of these 5 fetuses showed more activity within the samples from the dorsal aorta; in the other two the pulmonary and aortic samples were equally radioactive (table 1). In the one hour postpartum specimen the sample from the dorsal aorta was twice as active as that from the pulmonary artery; but in all of the pups older than one hour, a greater proportion of the injected radiomaterial appeared in the blood from the pulmonary artery. However, there was no marked decrease in the activity of the aortic samples until 9 hours postpartum. Considerable variation in the ratio of activity between the pulmonary and aortic samples appeared in the early postpartum pups. Variation was apparent throughout the remainder of the series (table 1); for example, in specimen no. 41 which was 6 hours old the ratio between the two blood samples was comparable to that of the fetuses. Also, the determinations for specimen no. 20, a three and one-half day old pup, were comparable to those obtained from pups two to 7 hours of age. Except for this variation

TABLE 1

Relative amounts of P^{32} recovered in blood samples from dorsal aorta and pulmonary artery

NO.	AGE	RATIO P^{32} DA [1]: PA [2]	NO.	AGE	RATIO P^{32} DA [1]: PA [2]
1	Late fetal	1.2: 1.0	12	37 hours	1.0: 196.0
2	Late fetal	1.0: 1.0	13	2 days	1.0: 249.0
3	Late fetal	2.0: 1.0	14	2 days	0.0:
4	Late fetal	1.0: 1.0	15	2 days	1.0: 316.0
5	Late fetal	1.7: 1.0	16	2½ days	1.0: 19.0
6	1 hour	2.1: 1.0	17	2½ days	1.0: 6.1
47	2 hours	1.0: 3.6	18	3½ days	1.0: 44.0
46	4 hours	1.0: 9.5	19	3½ days	1.0: 13.0
44	5 hours	1.0: 9.0	20	3½ days	1.0: 4.5
41	6 hours	1.0: 1.2	21	4½ days	1.0: 499.0
42	7 hours	1.0: 2.4	22	4½ days	1.0: 1496.0
35	7 hours	1.0: 9.0	23	5½ days	1.0: 322.0
36	7 hours	1.0: 4.0	24	5½ days	1.0: 115.0
7	9 hours	1.0: 77.0	25	5½ days	1.0: 210.0
8	9 hours	1.0: 37.0	30	6½ days	1.0: 4504.0
37	10 hours	1.0: 412.0	31	6½ days	1.0: 52.0
38	10 hours	1.0: 690.0	32	6½ days	1.0: 472.0
43	12 hours	1.0: 5.2	33	7 days	1.0: 1053.0
45	14 hours	0.0:	26	8½ days	1.0: 337.0
9	27 hours	1.0: 4000.0	34	12 days	1.0: 676.0
10	33 hours	1.0: 733.0	27	15 days	1.0: 814.0
39	34 hours	0.0:	40	18 days	0.0:
11	36 hours	1.0: 39.0	28	21 days	0.0:
			29	21 days	0.0:

[1] Dorsal aorta.

[2] Left pulmonary artery.

very little activity was detectable in the aortic samples from pups older than 12 hours.

Samples from the dorsal aorta of animals older than 15 days were non-radioactive, which indicates that the ductus arteriosus was physiologically non-functional. Comparable results were obtained from a few pups younger than 15 days; namely no. 45, a 14 hour specimen; no. 39, which was 34 hours of age; and no. 14, a two day postpartum pup.

Observation of the ductus arteriosus at the time of its exposure and during the experimental procedure, which was complete in from one to three minutes, revealed no gross changes within this brief period. This vessel was approximately isodiametric throughout its length in the late fetal and early postpartum specimens. However, in animals older than 7 hours the ductus was constricted near its point of juncture with the dorsal aorta.

Histologically the ductus arteriosus of the one hour and of the two hour postpartum specimens showed no evidence of anatomical closure (fig. 1). In pups from 4 to 12 hours of age partial constriction had occurred. Although the degree of closure varied within this age range, the more marked constriction was usually associated with the older specimen. The diameter of the ductus lumen in 7 hour animals was about one-fourth of that observed in the late fetal and one to two hour postpartum group (fig. 2). By 12 hours after birth the lumen of the ductus was markedly reduced (fig. 3). This reduction was produced by the contraction of smooth muscle in the ductus wall, which was most pronounced near the aortic junction. The degree to which the ductus was closed in animals 12 hours of age and older was less variable than in the 4 to 12 hour age group. The general picture in these was that of a persisting small slit-like opening, even in the most constricted part of the vessel, until anatomical cloure was complete (fig. 4). This final closure seemed to occur from about the 15th to the 18th day (fig. 5).

DISCUSSION

The physiological and anatomical findings of the present study indicate that in the dog the ductus arteriosus remains functional (though to a variable degree) for a considerable period of time following birth. It appears that there is a perceptible decrease in the flow of blood through the ductus in from one to two hours after delivery, but the more pronounced reduction of blood flow occurs at about 9 hours postpartum. In most specimens a very small amount of blood continues to traverse the ductus until about the 15th day when anatomical closure is usually complete. In a few animals the ductus flow ceased much earlier than 15 days, and in certain others the flow was considerable, even as late as three and one-half days postpartum. These, however, were exceptions and were not typical of the series. In a general way the size of the ductus lumen, as revealed by the histological study, was related to the amount of blood that was detected passing through the channel. However, after about the 9th hour postpartum this correlation did not always apply. From this time to the 15th day the histological picture of the ductus remained about the same; that is, markedly constricted but with a persisting slit-like opening. Thus, the anatomical variation was much less than the observed variation in blood flow.

It is possible that the considerable reduction of blood flow through the ductus arteriosus, which was observed to occur at about the 9th hour postpartum, is a phenomenon similar to what has been reported as the functional closure by Hamilton et al. ('37); Barclay et al. ('41); and by Kennedy and Clark ('41). According to these investigators, however, the closure was complete and occurred much sooner following birth. It is believed that these discrepancies can be explained in part by the differences in experimental procedures. The isotopic method employed in the present study makes possible the detection of even a trace of blood that might pass from the ductus arteriosus into the dorsal aorta. Actually the observed ratio of activity of the blood from the dorsal aorta at 9 hours postpartum as compared to that within the pulmonary artery is minimal (nos. 7 and 8, table 1) The ratios D.A.: P.A. in these two, which are 1:77 and 1:37 respectively, are relative and cannot be interpreted to mean that in the one the ductus flow is 1/37th or in the other that it is 1/77th of the flow in the left pulmonary artery. However, the ratios do mean that a considerable reduction in the ductus flow has occurred. It is questionable that the radiographic method of Barclay et al. or that the measurement of pressure changes within the heart ventricles, the method employed by Hamilton et al. ('41), would detect such a small amount of blood flow. Yet, the new evidence indicates that in the dog the ductus arteriosus continues to function for a considerable period following birth, although minimally and to a variable degree. This variation in the amount of blood flow through the ductus of specimens of the same or approximate ages appears to be in line with observations of Barclay et al. ('41), who observed that in the lamb the ductus closure may cause only a temporary cessation of flow, and subsequently at times may allow smaller quantities of the injected material to pass.

SUMMARY AND CONCLUSIONS

The relative amount of blood flow through the ductus arteriosus has been determined in late fetal and in postpartum pups up to three weeks of age. In addition, the ductus arteriosus of these pups has been studied histologically and the findings have been correlated with the physiological results. The following conclusions were drawn:

1. The ductus arteriosus remains functional (though to a variable degree) for a considerable period following birth.
2. Some reduction in the ductus flow occurs one to two hours after birth, but the more marked reduction occurs at about 9 hours postpartum.
3. A small slit-like lumen persists in the ductus arteriosus from about the 12th hour postpartum to the 15th to the 18th day, when anatomic obliteration is usually complete.

4. In most specimens a trace of blood can be detected passing through the ductus until the anatomical closure is completed.

LITERATURE CITED

BARCLAY, A. E., J. BARCROFT, D. H. BARRON AND K. J. FRANKLIN 1939 A radiographic demonstration of the circulation through the heart of the adult and the foetus and the identification of the ductus arteriosus. Brit. J. Radiol., *12:* 505–517.

BARCLAY, A. E., J. BARCROFT, D. H. BARRON, K. J. FRANKLIN AND M. M. L. PRICHARD 1941 Studies of the foetal circulation and of certain changes that take place after birth. Am. J. Anat., *69:* 383–406.

EVERETT, N. B. AND R. J. JOHNSON 1950 Use of radioactive phosphorus in studies of fetal circulation. Am. J. Physiol., *162:* 147–152.

GÉRARD, G. 1900 De l'obliteration du canal artériel. Les théories et les faits. J. Anat. Paris, *36:* 323–357.

HAMILTON, W. F., R. A. WOODBURY AND E. B. WOODS 1937 The relation between systemic and pulmonary blood pressures in the fetus. Am. J. Physiol., *119:* 206–212.

KENNEDY, A. J. AND S. L. CLARK 1941 Observations on the ductus arteriosus of the guinea pig in relation to its method of closure. Anat. Rec., *79:* 349–371.

PATTEN, B. M. 1946 Human Embryology, Blakiston, Philadelphia.

PLATE 1

EXPLANATION OF FIGURES

All of the sections illustrated are through the most constricted part of the ductus arteriosus.

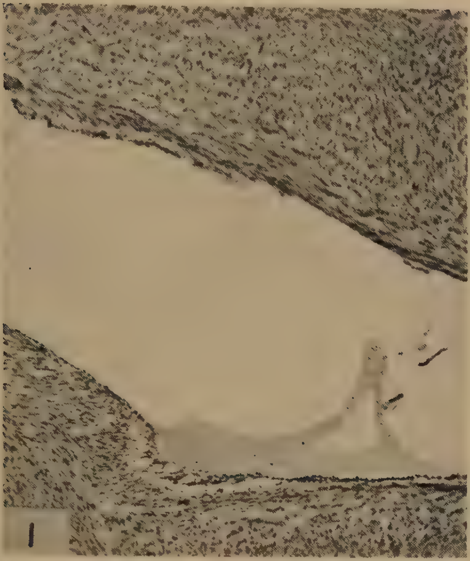
1 Ductus arteriosus of a one hour postpartum specimen. Longitudinal section. × 80.

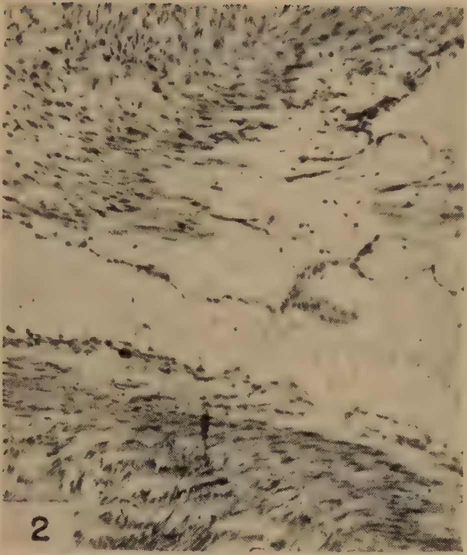
2 Section of the ductus arteriosus from a pup showing entry of the ductus into the dorsal aorta which is at the right of the figure. × 220.

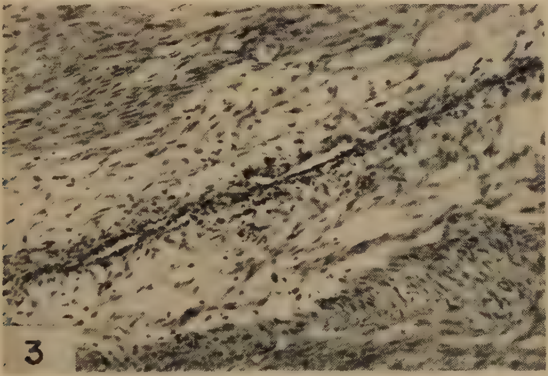
3 Oblique section of the ductus from a 12 hour specimen. Note mass of smooth muscle on either side of the lumen. × 220.

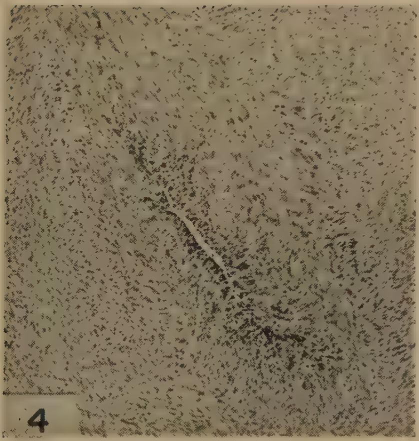
4 Oblique section of the ductus of a three and one-half day pup. × 80.

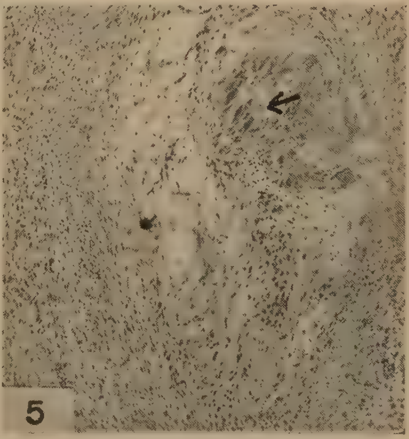
5 Transverse section of the ductus arteriosus from a 15 day specimen. Note at arrow that the ingrowth of tissue has almost completely obliterated the lumen. × 80.


1


2


3


4


5

PERICARDIO-PERITONEAL HERNIAE IN MAMMALS, WITH DESCRIPTION OF A CASE IN THE DOMESTIC CAT

CHARLES A. REED
College of Pharmacy, University of Illinois, Chicago, Illinois

TWO FIGURES

Diaphragmatic abnormalities with resulting unnatural migration of abdominal organs into a pleural cavity ("false" hernia) or bulging of an unruptured peritoneal sac through a weakened part of the diaphragm with displacement of the pleura ("true" hernia) are not as uncommon as once thought. On this subject there is an extensive literature for man and a growing one for other mammals.

In contrast, abdominal herniation into the pericardial cavity, or direct pericardio-peritoneal openings without herniation, are so exceedingly rare that such instances usually are not even mentioned in the extensive medical reviews of diaphragmatic herniae. Indeed, only 22 cases of this type were found (table 1) in a review of the literature. (This review may be incomplete with regard to foreign journals.) The 23rd, here described, was found in a laboratory cat when a puzzled student claimed that the specimen being dissected had no greater omentum.

The animal was a large, fat, female, domestic cat, procured dead and injected from a biological supply house. At the time of death she had recently given birth to kittens, the mammary glands had been fully functional, and in general there was no indication that the defect to be described had had any deleterious results.

A round aperture, 3.5 cm in diameter, was found in the ventral asect of the diaphragm, directly to the right of the

TABLE 1

Pericardio-peritoneal herniae

AUTHOR	ANIMAL	DATE	AGE	SEX	TYPE	HERNIATED STRUCTURES	ORIGIN
1. de Cardenal[1]	Man	1903	58	M	False	Great omentum, loop of transverse colon	Congenital
2. Martland[1]	Man	1909	70	M	False	Great omentum, loop of transverse colon	Congenital
3. Keith[1]	Man	1910	50	M	False	Part of great omentum	Possibly traumatic
4. Keith[1]	Man	1910	47	M	False	Part of liver	Possibly traumatic
5. Keith[1]	Man	1910	Newborn	?	True	Part of liver	Congenital
6. Hedblom	Man	1925	?	?	?	?	?
7. Taillens[2]	Man	1934	2 mo.	?	?	Pyloric half of stomach, duodenum, part of left liver, and most of the colon	Prob. congenital
8. O'Brien	Man	1939	63	M	False	None	Congenital
9. Casey and Hidden	Man	1944	Newborn	?	False	None	Congenital
10. McCrory and Bunch	Man	1947	Newborn	M	False	Superior surface of right lobe of liver	Congenital
11. McCrory and Bunch	Man	1947	Newborn	M	False	Right lobe of liver	Congenital
12. Wilson, Rumel and Ross	Man	1947	7 wk.	M	False	Three-fourths of the small intestine	Congenital
13. Gohier[3]	Dog	1811	?	?	Prob. False	Small part of the liver	?
14. Antonio	Dog	1901	?	?	False	Greater omentum	Congenital
15. Petit[1]	Dog	1902	?	?	False	Coils of small intestine	Congenital
16. Joest[1]	Dog	1904	Old	?	False	Greater omentum	Congenital
17. Stoeber[1,3]	Dog	1912	?	?	? True	Part of liver	Congenital
18. Waldmann	Cat	1910	1 yr.	?	True	Most of the abdominal viscera	?
19. Bru	Cat	1923	6 mo.	?	False	Supernumerary liver and gallbladder	Prob. congenital
20. Brisco	Cat	1928	Adult	?	True	Gallbladder, part of liver, and small part of greater omentum	?
21. Reed	Cat	1951	Adult	F	False	Gallbladder, greater omentum, and left, caudate, and cystic lobes of liver	Prob. congenital
22. Thomson	Rabbit	1901	Adult	?	True	Part of liver	?
23. Forgeot[1,3]	Donkey	1903	?	?	False[1] True[3]	Part of liver	?

[1] Quoted by O'Brien ('39).
[2] Quoted by Wilson, Rumel and Ross ('47).
[3] Quoted by Bru ('23).

falciform ligament, which was normal in development but displaced anteriorly. The ruptured portion of the diaphragm that typically would have closed this opening was present as a loose flap of nonmuscular tissue. Through the opening and into the enlarged pericardial cavity protruded the whole of the greater omentum ventrally, and dorsally the following parts of the liver: the left lobe, the caudate lobe, and the part of the cystic lobe containing the gall bladder. The remainder of the cystic lobe, not normally a separate mass, and the right lobe of the liver, considerably hypertrophied, lay outside the pericardial cavity, as did all of the other abdominal viscera. The parts of the liver in the pericardial cavity were globular in shape and definitely reduced in size but the biliary ducts and circulatory vessels appeared normal in configuration, even if somewhat displaced.

The enlarged pericardial cavity (8.5 cm long, 5.0 cm across, and 4.0 cm deep, as compared to normal dimensions of 4.0 by 3.0 by 2.0 cm) had expanded at the expense of the pleural cavities, so that the lungs were small and displaced dorsally; the left posterior (mediastinal) lobe of the right lung was particularly small.

The heart had been compressed forward and twisted, so that the normal ventral aspect of the ventricle pressed against the left pericardium. The right atrium, however, retained its usual position and thus superficially appeared to be a structure separate from the ventricle. The left atrium was remarkably small. Thus the living heart was certainly under continuous pressure from the encroaching liver and was distorted as well as displaced. However, the healthy appearance of the cat speaks for efficient cardiac function during the life of the animal, despite the anatomic abnormality.

The particular hernia here described is through that part of the diaphragm which develops from the transverse septum. The evidence available would indicate that the septum developed typically but was never invaded by muscle, and that at some time prior to birth a true hernial sac ruptured forward into the pericardial cavity. This hernial sac then

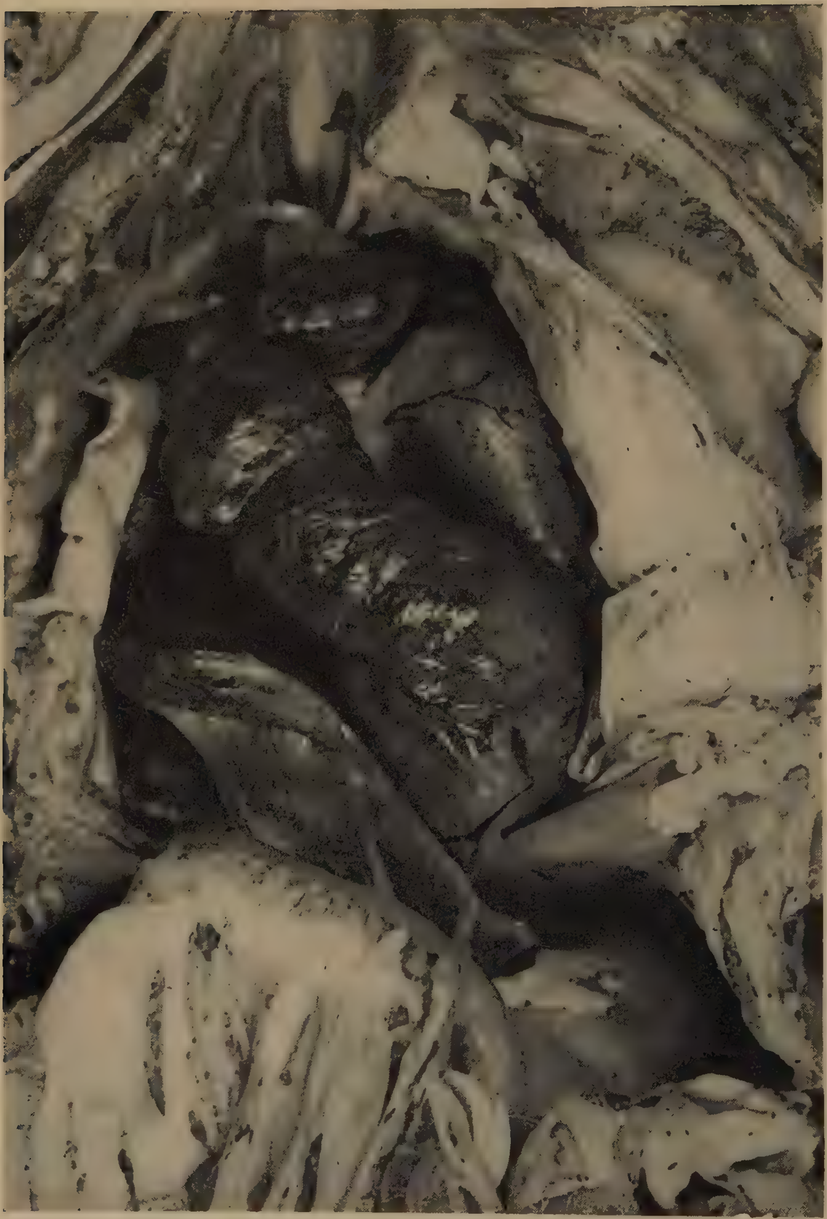


Fig. 1 Opened pericardial cavity of abnormal cat, ventral view, showing part of the liver lying in the pericardial cavity and heart displaced forward; the greater omentum, also present in the pericardial cavity in life, has been removed.

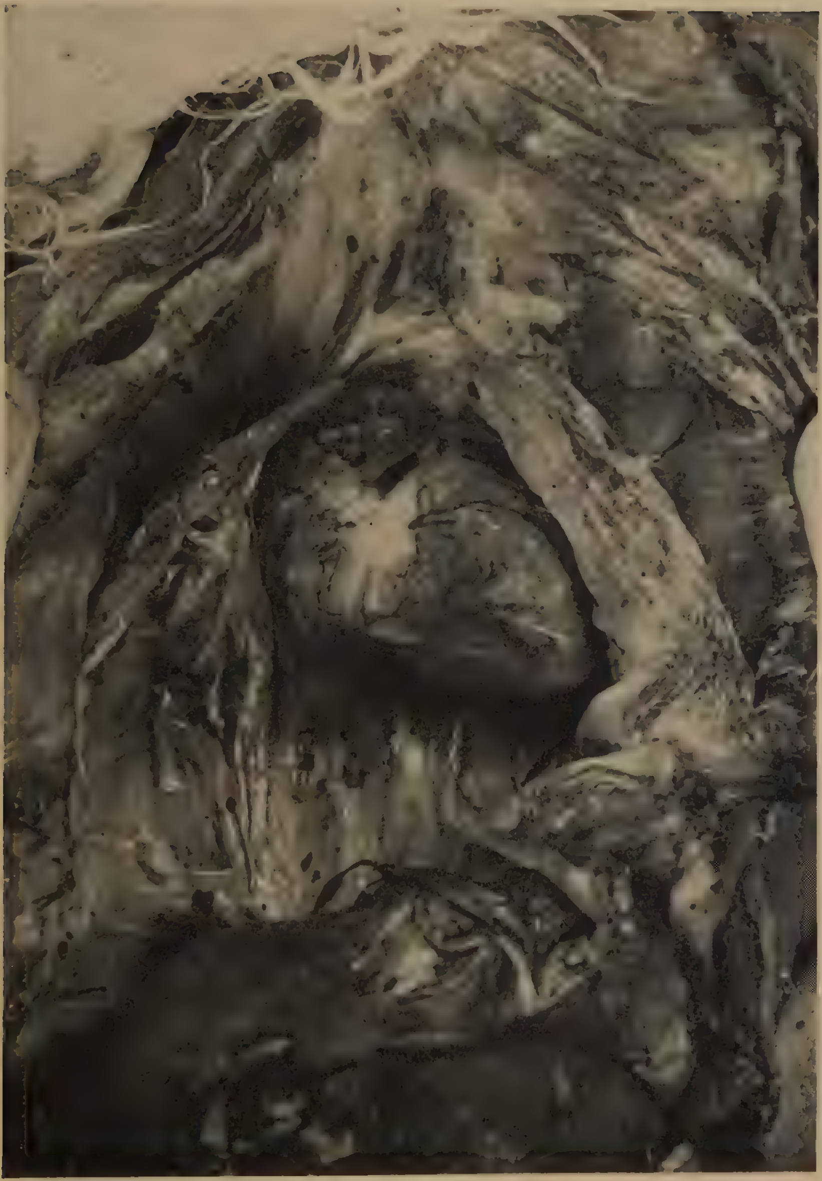


Fig. 2 Same as figure 1, but with the liver removed from the enlarged pericardial cavity; the ventricle and the right atrium appear to be separate organs.

secondarily broke to produce a false hernia; a remnant of this sac, to which the falciform ligament was attached, was still present.

In both the cat and the dog the diaphragm, including the part derived from the septum transversum, comes to lie so far posteriorly that the pericardial cavity is no longer adjacent to it, and, since this condition is established before birth, any traumatic injury at or following birth leading to a diaphragmatic rupture will result in pleuro-peritoneal hernia. In man, on the contrary, where the diaphragm forms one wall of the pericardial cavity, the chance of pericardioperitoneal hernia of traumatic origin is always present, although the record would indicate an extreme rarity of occurrence.

Considering the large number of human autopsies, and especially of autopsies of the still born and of those dying soon after birth, as contrasted with the situation in other mammals, it is surprising to find this type of hernia so well represented in cats and dogs (9 cases, all adult) as compared with man (12 cases, 5 in new-born or infant dead). There is, too, the further factor that there is less likelihood of anatomical abnormalities in domestic animals being reported even when found.

LITERATURE CITED

Brisco, G. 1928 Hernia through the central tendon of the diaphragm (cat). J. Anat., *62:* 224–226.

Bru, P. 1923 Foie surnuméraire intrapéricardique englobant le coeur. Rev. Veter., *75:* 580–587.

Casey, A. E., and E. H. Hidden 1944 Nondevelopment of the septum transversum with congenital absence of the anterocentral portion of the diaphragm and of the suspensory ligament of the ductus venosus and a pericardioperitoneal foramen. Arch. Path., *38:* 370–374.

Hedblom, C. A. 1925 Diaphragmatic hernia: A study of 378 cases in which operation was performed. J. A. M. A., *85:* 947–953.

Keith, A. 1910 Remarks on diaphragmatic herniae. Brit. Med. J., 1910 (2): 1297–1298.

Martland, H. S. 1909 A case of congenital false diaphragmatic hernia. J. A. M. A., *52:* 1574–1576.

McCRORY, W. W., AND R. F. BUNCH 1947 Omphalocoele with diaphragmatic defect and herniation of the liver into the pericardial cavity. J. Pediat., *31:* 456–464.

O'BRIEN, H. D. 1939 Pericardio-peritoneal communication. Description of a rare type of diaphragmatic hernia. J. Anat., *74:* 131–134.

THOMSON, A. 1901 A remarkable condition in a rabbit simulating the occurrence of a double heart. J. Anat. and Physiol., *35:* xvi–xix.

WALDMANN, J. 1910 Ueber einen eigenthümlichen Fall von Hernia intestinalis diaphragmatico-pericardialis bei ein Kater. Jahresb. ü. d. Leist. a. d. Geb. d. Vet.-Med., *29:* 188.

WILSON, A. K., W. R. RUMEL AND O. L. ROSS 1947 Peritoneo-pericardial diaphragmatic hernia: Report of a case in a newborn infant successfully corrected by surgical operation with recovery of the patient. Am. J. Roentgen. and Rad. Ther., *57:* 42–49.

THE TRANSFER FUNCTION OF CERTAIN CELLS IN THE WALL OF THE GRAAFIAN FOLLICLE AS REVEALED BY THEIR REACTION TO PREVIOUSLY STAINED FAT IN THE CAT

R. M. WOTTON AND P. A. VILLAGE
The Department of Biological Sciences, Duquesne University, Pittsburgh, Pa.

THREE FIGURES

INTRODUCTION

Wilson ('47) and others have suggested that the nutrition of the oöcyte, during a major portion of its development, is associated closely with the activity of certain accessory nurse cells. In the cat, and other mammals, these are separated from the egg cell by the barrier of the zona pellucida. This layer, however, is penetrated by protoplasmic connections which maintain contact between the enclosed ovum and its accessory cells in the follicle wall and which may serve as channels for the transport of nutritive materials into the oöcyte. It would be desirable to test this predication experimentally by administering some food substance tagged in such a way that its passage could be followed to the ovum.

Gage and Fish ('24), Wotton and Zwemer ('39), and Zwemer and Wotton ('44) hav shown that previously stained fat can be fed, and subsequently traced through the body. Due to the differential staining and other physical properties of lipids, permanent microscopical preparations can be made by the gelatin imbedding and frozen section technique (Zwemer, '33), showing the distribution of previously stained fat in the tissues. Zwemer and Wotton ('44) employed such

THE ANATOMICAL RECORD, VOL. 110, NO. 2
JUNE 1951

a procedure in their study of kidney function during fat excretion, and the present experiments apply a similar approach to a study of the function of the protoplasmic channels through the zona pellucida.

MATERIALS AND METHODS

The ovaries from 24 young kittens and two mature cats were used in this study. These animals were fed cod liver oil saturated with Sudan IV through a small rubber tube attached to the tip of a glass hypodermic syringe. Gentle pressure on the plunger of this syringe forced the test meal of about 5 cm^3 into the stomach.

Following an interval of one to two hours each animal was sacrificed and immediately dissected. The ovaries with the adjacent parts of the fallopian tubes were fixed for 24 hours in 10% pyridine formalin (Burke, '37).

Tissues were embedded in gelatin and sectioned on the freezing microtome. The mounted sections were immersed in a solution of Sudan IV to intensify the color already present in the previously stained fat in the tissues, and counterstained in Harris' hematoxylin for nuclear differentiation. For comparison others were left completely unstained. All slides were mounted in glychrogel (Wotton and Zwemer, '35).

As a check on the formalin fixation, several ovaries were preserved in Champy's osmic-bichromate-chromic acid mixture, and sectioned by the paraffin technique. The blackening of the fat with osmic acid was sufficient to mark the oil droplets (Glick, '49), while staining with eosin was helpful in bringing out cytoplasmic structures.

In addition to the slides prepared from the tissues of the animals employed in this investigation, sections of ovaries from a series of cats used in a former study on fat absorption in the intestine (Wotton and Zwemer, '39) were reviewed. This brought to 40 the number of kittens observed for a reaction to fat by the ova and their accessory cells.

OBSERVATIONS

The accessory nurse cells

The accessory cells in the follicle wall surrounding the ova appear to be of two morphologically different types. The most commonly seen cells are oval in shape, with an elongated process terminating in a slight swelling upon the outside of the zona pellucida. From this enlargement a filose projection of cytoplasm could be observed passing through an aperture in the zona into the oöcyte (fig. 2). The other are flat, closely adherent, and spread out upon the surface of the zona with several lobose, protoplasmic elongations through this membrane into the egg cell beneath (fig. 3). While it was usual for one accessory cell to occupy with its cytoplasmic extension a single opening through the zona pellucida, often two or more cells were seen to employ one aperture in common for this purpose.

Fat droplets were observed in the accessory nurse cells, and more frequently they were situated in their blunt terminations which projected into the oöcyte. Fat drops with two lobes, one lying just inside the cytosome of the ovum and still connected by a narrow strand to the other part remaining within the projection from the follicle cell were seen (fig. 2).

The ova

In each section studied, stained oil was observed in almost all ova inclosed in vesicular or Graafian follicles. These cells were ovoid in section and contained a lobulated nucleus with a prominent, vesicular plasmosome and several karyosomes. Large clear vacuoles were seen which tended to arrange the cytoplasm into a peripheral zone from which thick strands passed inward to the nucleus. Granules and spheroids of lipid were observed in this peripheral zone directly beneath the cell membrane; and also densely packed in the pathways approaching the nucleus (fig. 1). In many ova certain oil drops could be detected in very close contact with the nuclear membrane.

None of the follicles observed in this study showed signs of atresia, such as the resorption of the liquor folliculi and epithelial destruction accompanied by karyolysis.

DISCUSSION

In the early development of a new individual, the ovum has a dual function, for, as well as furnishing a genetic component, it supplies some food substances for the cells of the future embryo. The nutritive materials are absorbed and retained in the cytosome by the oöcyte during its growth within the follicle. This storage of food chiefly accounts for the relatively large size of the mature egg cell. The acquisition of these materials seems to be carried out through the intermediary activity of the accessory follicle cells, acting as transfer agents across the barrier of the zona pellucida.

These cells, situated in the wall of the Graafian follicle, pick up fat as droplets released from the capillary circulation and transport them by way of their protoplasmic projections through the apertures in the zona pellucida into the ovum. Once within the oöcyte the oil droplets appear to pass along the cytoplasmic pathways from the peripheral zone toward the circumnuclear region (fig. 1). The close association between certain fatty globules and the nuclear membrane seemed to suggest some reciprocal activity (Boyd, '35). Histologically no change could be detected in the previously stained cod liver oil during its passage through the accessory nurse cells; and presumably the physiological activity of such cells, as far as lipids are concerned, is confined mainly to the function of its physical transfer from the tissue spaces into the oöcyte.

Our observations on the appearance of constricted oil droplets seen at the tips of the projections from the follicle cells, where they come in contact with the periphery of the cytosome of the ovum, clearly demonstrate the existence of an interface or membrane between these cells. Singer and Zwemer ('34), and Zwemer, Wotton and Norkus ('38), Wotton and Zwemer ('39), and Zwemer and Wotton ('44) have reported similar pictures for the transfer of lipid in such hourglass shape in and out of cells. The constriction is caused by the compression of the plastic oil as it squeezes through a tiny pore in the cell membrane. Thus it would seem that the question of cytoplasmic continuity (Wilson, '47) between these nurse cells and their oöcyte in the cat can be answered in favor of a close contact between the two cell surfaces across which fats, and presumably other food substances, are transferred.

SUMMARY

In a series of 40 kittens the cellular channels through the zona pellucida by which the oöcyte may receive food substances were studied. They were found to be projections belonging to two types of accessory nurse cells situated in the follicle wall.

By following the path of previously stained cod liver oil from the blood capillaries into the ovum the transport function of these accessory cells for this substance, and presumably for other materials needed by the ovum, was established.

LITERATURE CITED

Boyd, E. M. 1935 The relation of lipid composition to physiological activity in the ovaries of pregnant rabbits. J. Biol. Chem., *108:* 607.

Burke, F. V. 1933 The pH of formalin. A factor in fixation. Am. J. Path., *9:* 915–920.

Gage, S., and P. A. Fish 1924 Fat digestion, absorption and assimilation in man and animals as determined by the dark-field microscope, and a fat soluble dye. Am. J. Anat., *24:* 1–77.

Glick, D. 1949 Techniques of Histo- and Cytochemistry. Interscience Publishers, Inc., New York. 68.

Singer, E., and R. L. Zwemer 1934 Microscopic observations of the structural changes in the adrenal gland of the living frog under experimental conditons. Anat. Rec., *60:* 183–187.

Wilson, E. B. 1947 The Cell in Development and Heredity. The Macmillan Co., New York. 3 ed.: 331–338.

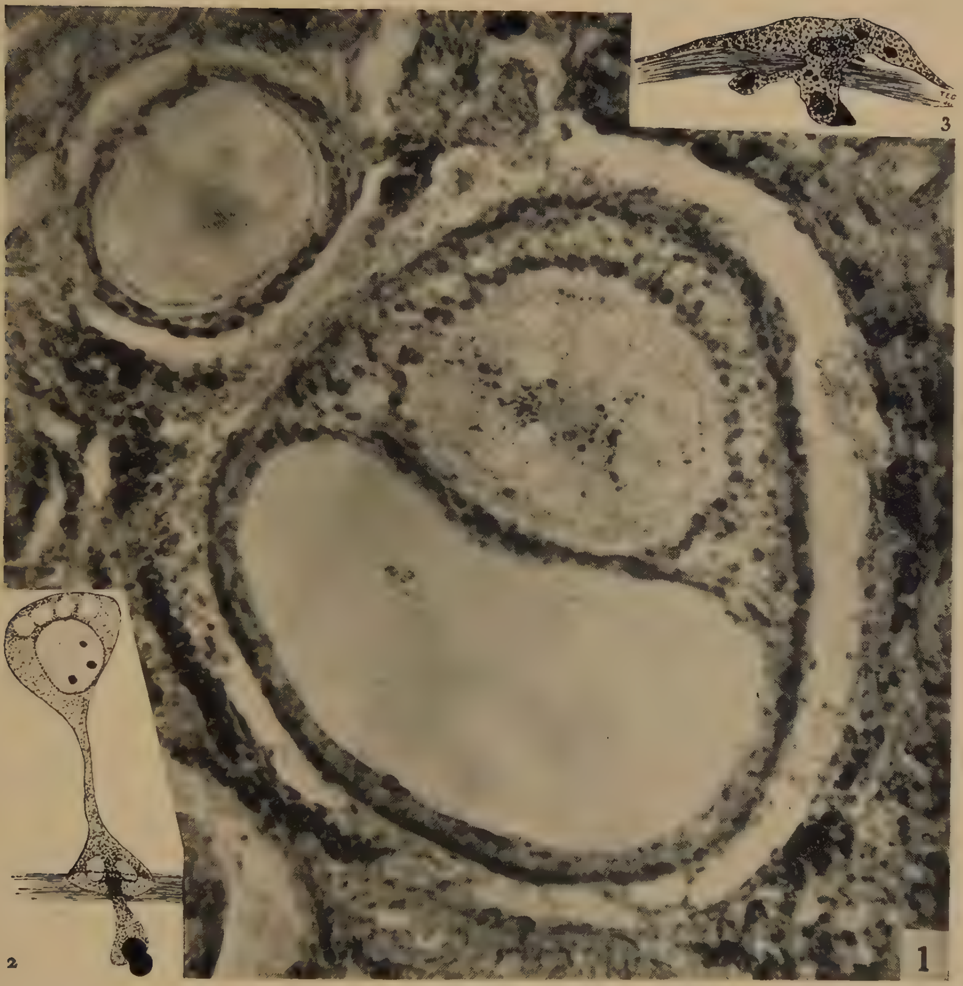
Wotton, R. M., and R. L. Zwemer 1935 A note on ''Glychrogel'' mounting solution. Stain Tech., *10:* 21–22.

——— 1939 Studies on direct and visible ingestion of fat by differentiated body cells of the cat. Anat. Rec., *75:* 493–507.

ZWEMER, R. L. 1933 A method for studying adrenal and other lipoids by a modified embedding and mounting technique. Anat. Rec., *57:* 41–44.

ZWEMER, R. L., AND R. M. WOTTON 1944 Fat excretion in the guinea pig kidney. Anat. Rec., *90:* 107–114.

ZWEMER, R. L., R. M. WOTTON AND M. G. NORKUS 1938 A study of cortico-adrenal cells. Anat. Rec., *72:* 249–263.



1 Graafian follicle showing fat inclusions. × 200.

2 Camera lucida drawing of nurse cell type found in the wall of Graafian follicle. × 3000.

3 Camera lucida drawing of second nurse cell type. Compare with figure 2. × 3000.

THE ZONE OF MINERALIZATION AND THE ACTION OF ALIZARIN IN PREVENTING ITS APPEARANCE IN BONES GROWING IN TISSUE CULTURE

GEORGE H. PAFF, ARMAND W. ANGULO AND F. C. EKSTEROWICZ
Division of Anatomy, Hahnemann Medical College and Hospital,
Philadelphia, Pennsylvania

FOUR FIGURES

INTRODUCTION

Recently we described experiments in which alizarin red S was used to stop selectively the growth of bone in tissue culture (Paff and Eksterowicz, '50). Since then our work has been extended to a study of the effect of the dye on developing bones at a stage when no bone tissue is present. It was desired to learn if alizarin red S would prevent the beginning of osteogenesis while leaving other tissues unaffected in their growth. The answer to this question, coupled with findings on the earliest indication of activity leading to bone formation is presented below.

CLARIFYING REMARKS

The process of osteogenesis as it occurs in vitro is a relatively simple one compared to that seen in vivo. In tissue culture, one sees only the earliest stages of bone formation moving at a painfully slow rate. This fact was impressed upon us when we began to use alizarin to mark the newly formed bone tissue. It was expected that alizarin would stain only the primitive spicules of bone. Instead it was found that the first indication of the beginning of osteogenesis is the appearance of an alizarin receptive bone which stains diffusely red (figs.

1, 2, 3). This zone invariably precedes the formation of spicules by several days and spicules never form anywhere but within it. It is probably similar to the region of calcified cartilage which precedes actual spicule formation in endochondral bone formation in vivo. In tissue culture, however, it is a well circumscribed area which can be flaked off the underlying cartilage. This observation on long bone growth in tissue culture is more closely related, we believe, to that of Bevelander and Johnson ('50) on flat bone growth in vivo, i.e., "Mineralization appears to take place to some extent very early in the formation of bone, even before the spicule is well organized."

In the following description the phrase, zone of mineralization, will be used in referring to the collar-shaped, spicule-free, alizarin receptive region. Its appearance indicates that the process of osteogenesis is beginning.

CHOICE OF MATERIALS

Since the growth rate of bone tissue in vitro is very slow compared to the rate in vivo, the wise choice of a bone can save much time. We sought a bone in the pre-osseous stage which was just on the verge of depositing material which could be detected with alizarin red S. After preliminary experiments with more than 200 pairs of femurs, tibias, fibulas, and metatarsals of various ages, the fibula of the chck embryo at 8 days of incubation was finally chosen. In routine material the fibula at this age lies at the boundary line between the pre-osseous and osseous stages. There is just enough variation in the degree of development that some of the fibulas contain a zone of mineralization, others do not.

Because of the varying degree of development, it was necessary to isolate and culture 36 pairs of fibulas to obtain the series represented in table 1. We selected fibulas in which a zone of mineralization had not yet formed. These fibulas contained only two types of tissue, namely, young cartilage and fibrous connective tissue. The latter was represented by the perichondrium and tabs of tissue adherent to it. By using the

TABLE 1

Data on 13 pairs of fibulas

See text for description

NUMBER OF PAIR	ALIZARIN		CONTROL	
	Length at start	Length after 5 days	Length at start	Length after 5 days
#1				
Total length	3.15	3.75	3.00	3.75
Zone mineralization	.	...	.	0.40
#2				
Total length	2.80	3.60	2.85	3.75
Zone mineralization	.	. .	...	0.50
#3				
Total length	2.50	3.60	2.65	3.25
Zone mineralization	. .	...	...	0.25
#4				
Total length	3.00	3.70	2.95	3.65
Zone mineralization	...		...	0.70
#5				
Total length	2.90	3.75	2.90	3.60
Zone mineralization	. .	.	.	0.45
#6				
Total length	3.10	3.70	3.40	4.30
Zone mineralization	.	.	. .	0.60
#7				
Total length	3.00	3.85	2.85	3.50
Zone mineralization	...	.	...	1.15
#8				
Total length	3.20	4.30	3.15	4.35
Zone mineralization	...	. .	...	1.25
#9				
Total length	3.00	3.90	3.00	4.00
Zone mineralization	. .	...	...	1.75
#10				
Total length	2.60	3.35	2.50	3.35
Zone mineralization	. .	...	...	1.20
#11				
Total length	3.00	3.65	3.10	3.55
Zone mineralization			...	0.40
#12				
Total length	2.85	3.60	2.90	3.75
Zone mineralization	...	...	. .	0.50
#13				
Total length	3.00	3.75	3.10	3.75
Zone mineralization	.	.	...	0.25
Ave. total length	2.93	3.71	2.95	3.76
Ave. zone mineralization		.	. .	0.72

fibula it was possible to obtain results in 5 days which, in the case of femurs, sometimes require two to three weeks.

TECHNIQUE

The fibulas were cultured on the surface of clotted hanging drops, one fibula to a drop. The controls were grown on equal parts of blood plasma and embryonic extract; the experimental bones on equal parts of the same ingredients containing alizarin red S in the proportion of one drop of a Seitz-filtered saturated watery solution of the dye to 59 drops of embryonic extract. Experiments were terminated at the end of 4 or 5 days. At this time the controls were stained with alizarin by adding one drop of alizarin-embryonic extract to each preparation. Measurements of the total lengths of the bones and lengths of the zones of mineralization were taken.

The alizarin technique was also used in studying the zone of mineralization in the long bones of 9 to 10 day old chick embryos. In addition we employed a modification of the procedure described by Lillie ('48) as a control for the technique of visualizing phosphatase in situ. Both the alizarin stained and unstained members of a pair of bones were placed in 0.1% $Ca(NO_3)_2 \cdot 4H_2O$ for two hours at room temperature and then into 5% $Ag(NO_3)_2$ under a strong light. After the zone of mineralization had become black, the preparations were washed for at least one hour in distilled water and finally mounted in glycerin-jelly.

Some of the cultures were subjected to the silver technique without preliminary fixation, others were fixed in either 10% formalin or 95% alcohol.

OBSERVATIONS

The more pertinent results obtained with 13 pairs of 8 day old fibulas are given in table 1. The total length of the fibulas, the absence or presence of a zone of mineralization, and the length of the zone when present are given for both the alizarin treated and control members of each pair.

The close agreement in total lengths of the alizarin treated and control bones should be noticed. After 5 days in vitro, the average increase for the bones growing on media containing alizarin was 0.78 mm (2.93 to 3.71 mm). During the same time the controls had increased 0.81 mm (2.95 to 3.76 mm).

In contrast to the agreement in total length between the experimental and the control bones, the clean cut difference between the two groups as to the presence of a zone of mineralization should be noticed. Since fibulas without such a zone were selected originally, it is obvious that none of the bones will have it at the start of the experiment. This is indicated in table 1 by dashes. At the end of 5 days of growing in tissue culture, however, it should be noticed that, whereas all of the controls developed a zone of mineralization, not one of the bones growing on alizarin did so. The lengths of the zones of mineralization which developed in the controls varied from 0.25 to 1.75 mm. The average was 0.72 mm.

As for the connective tissue, a halo of new growth always developed and there was no evidence that it was affectd by the dye. Apparently the degree of development of the halo depended on the amount of perichondrium and the amount of connective tissue adherent to it at the time the fibula was planted in tissue culture. This agrees with our observations on older bones (Paff and Eksterowicz, '50).

In seeking a suitable bone for study, an interesting fact concerning the visualization of the zone of mineralization became evident, i.e., fixation can lead to a marked reduction in its size. As a base-line, we used the size of the zone visualized in living bones by the direct action of alizarin. To accentuate the zone stained by the dye, the silver technique was used. A black precipitate forms in the exact area of alizarin staining (fig. 3). If, however, the bones are fixed in 10% formalin and washed before being stained with alizarin and treated with the silver technique, a marked reduction occurs in the extent of the zone of mineralization (fig. 4). This reduction may vary from total loss of the zone in very young bones to a loss apparent only at the ends of the zone in older bones. Although we did not explore a range of fixatives, the effect is probably due to the solvent action of a watery fixative since fixation in 85% alcohol does not affect the size of the zone.

DISCUSSION

The action of alizarin in completely preventing the earliest appearance of osteogenetic activity in tissue culture offered theoretical evidence that the dye should be able to inhibit the growth of bone in vivo. In practice this has, at least to the present, proved to be a false hope. We have been unable, by using subcutaneous alizarin pellets or intraperitoneal injections to alter the growth rate of bone in relationship to the growth rate of young rats. It is possible that the necessary concentration of alizarin cannot be achieved or maintained by the methods employed. It may be that by use of an air ejector (Hingson and Hughes, '47), one could raise the dye concentration in the immediate area of a bone such as a metatarsal and thus stop osteogenesis locally. Such proof must await further investigation.

In tissue culture, the variation in the size of the zone of mineralization following fixation may be explained on the following basis. When first deposited the organic salts are probably unbound or at least very loosely bound to the organic component of the tissue. If, now the bone is stained with alizarin, the inorganic material is visualized and is loosely bound in situ by formation of a lake. If, however, the bone is placed in a watery fixative before staining, the fixative produces a decrease in the amount of mineralization because of its solvent and washing actions. The older part of the zone of mineralization is much more resistant to removal of inorganic material indicating that in this region, binding is more secure.

Since the reaction involving alizarin is concerned primarily with calcium, the silver technique is an important complement. The black precipitate obtained with the silver procedure is silver phosphate. Thus a more complete picture of the disposition of salts in the zone of mineralization is obtained.

Finally, on the basis of these results, we should like to hazard the guess that peritoneal injections of alizarin red S in young animals, followed by free hand sections of fresh rapidly growing bones, followed by treatment with $Ca(NO_3)_2 \cdot 4H_2O$ and $Ag(NO_3)_2$ would lead to interesting results on the mineralization of prespicule areas in both endochondral and intramembranous areas of ossification in vivo. (See Bevelander and Johnson, '50.)

SUMMARY AND CONCLUSIONS

Isolated embryonic chick long bones in the preosseous stage of development behave as follows when grown on tissue culture media containing alizarin red S: First, a zone of mineralization fails to develop and second, the cartilage and connective tissue continues to grow in uninhibitive fashion.

In very young living bones, the zone of mineralization is an alizarin receptive area. Its visualization is enhanced by a silver technique. If fixation precedes treatment with alizarin and silver, the zone is less extensive. This indicates that calcium and phosphate are lost by fixation and points the way to a method of retaining these substances in a critical location for study purposes.

LITERATURE CITED

BEVELANDER, G., AND P. L. JOHNSON 1950 A histochemical study of the development of membrane bone. Anat. Rec., *108:* 1–22.

HINGSON, R. A., AND J. G. HUGHES 1947 Clinical studies with jet injection; new method of drug administration. Anesth. and Analg,. *26:* 221–230.

LILLIE, R. D. 1948 Histopathologic technic. Blakiston Co., Philadelphia.

PAFF, G. H., AND F. C. EKSTEROWICZ 1950 The selective stoppage of bone growth in tissue culture. Anat. Rec., *108:* 45–56.

PLATE 1

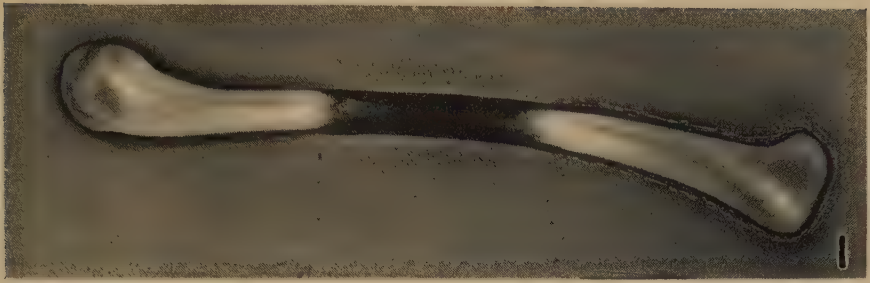
EXPLANATION OF FIGURES

1 Metatarsal from 9 day old chick embryo, cultured for 5 days on hanging drop. Stained witoh alizarin followed by silver technique. The zone of mineralization is black. No trabeculae were present. Drawing, × 19.

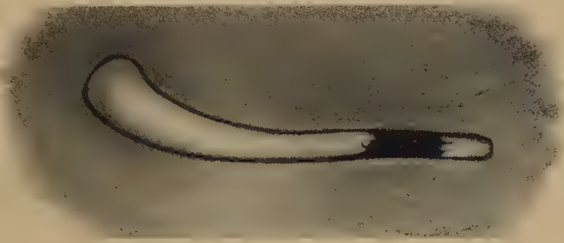
2 Fibula from 8 day old chick embryo, cultured for 5 days on alizarin-free medium. Stained with alizarin followed by silver technique. The zone of mineralization is black. (The mate to this bone had been grown on media containing alizarin and no zone of mineralization had developed.) No fixation. Drawing, × 16.

3 Zone of mineralization from an 8 day fibula grown for 5 days in vitro, stained with alizarin, treated with silver technique and mouned in glycerin-gelatin. No trabeculae are present. The clear spaces within the zone are areas occupied by cells. No fixation. Drawing. × 108.

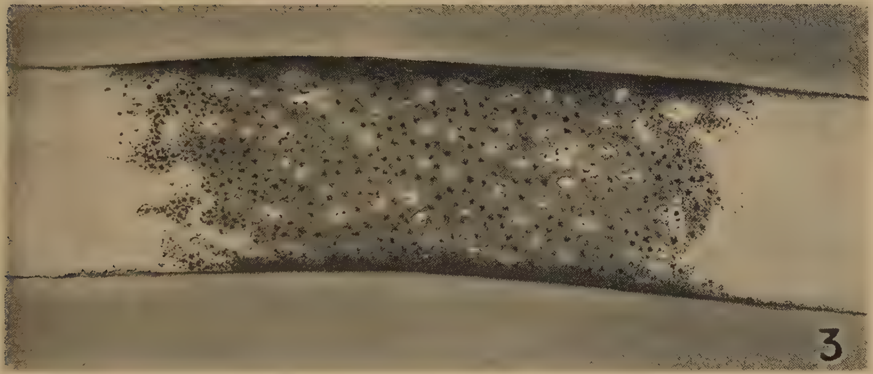
4 Zone of mineralization from an 8 day fibula, the mate to the bone shown in figure 3, grown for 5 days in vitro, fixed in 10% formalin, washed in water, and treated with the silver technique. Note the almost complete loss of mineralization. Drawing, × 108.



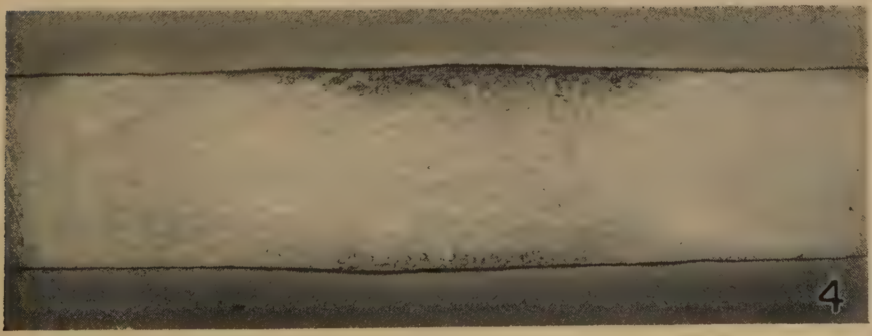
1



2



3



4

WEIGHTS OF THYROID AND PARATHYROID GLANDS OF NORMAL MALE DOGS[1]

R. M. MULLIGAN AND K. C. FRANCIS
Department of Pathology, University of Colorado, School of Medicine, Denver

THREE FIGURES

Precise data on the weights of the thyroid and parathyroid glands of normal dogs were not found in a survey of the literature, which is rather meager with regard to normal anatomic details of the endocrine glands of the dog as pointed out in a previous communication (Francis and Mulligan, '49). Consequently, the recording of the weights of these glands as determined in a series of 40 normal male dogs could be of some assistance in the assessment of variations in them in experimental procedures.

MATERIALS AND METHODS

Of 40 normal male mongrel dogs (6.8 to 33 kg) from the original series of 55 (Francis and Mulligan, '49), both the thyroid and parathyroid glands of 30, only the thyroid glands of 5, and only the parathyroid glands of 5 were available for study. Weight data for 35 thyroid glands and for 86 parathyroid glands were obtained (figs. 1 and 2). Of the 40 mongrel dogs, 19 had a predominant strain of shepherd and 15 of terrier with the remaining 6 miscellaneous or unspecified. After the skeletal muscles overlying the thyroid and parathyroid glands were cut away, the larynx, the proximal ends of the trachea and esophagus, and the glands in situ were removed in block and fixed in Zenker's fluid, washed in water, and stored in 70% ethyl alcohol. The two lobes of the thyroid

---

[1] This investigation was aided by a grant from the Continuing Research Fund of the University of Colorado, School of Medicine.

gland, the attached parathyroid glands, and the immediately adjacent soft tissues were then dissected free and placed in 70% ethyl alcohol, within which the parathyroid glands loose in the capsule or partially imbedded in the surface of the thyroid lobes were carefully separated and freed of supporting soft tissue. The thyroid lobes were trimmed in a similar manner and then were weighed on a torsion balance to the nearest 0.01 gm. The parathyroid glands were weighed on an analytical balance to the nearest 0.1 mg.

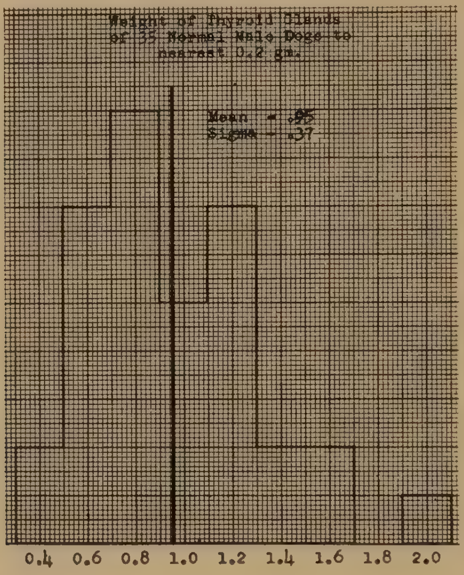


Figure 1

RESULTS

Among 35 pairs of thyroid lobes weighed, the variation between lobes was 0.1 gm or less in 25 dogs, 0.2 gm or less in 4, between 0.22 and 0.78 gm in 4, and identical in two. The weight of the entire thyroid gland ranged between 0.35 gm and 2.03 gm with a mean of 0.95 gm (fig. 1). The sigma was 0.37, the sum of the weights of the 35 glands was 33.40, and the sum of the squares of the weights was 36.4862. By dividing the 35 dogs into the three age groups defined on the basis of the development of costochondral junctions, testes, and prostate glands (Francis and Mulligan, '49), the 17 adults had an average thyroid weight of 1.10 gm, the 10 adolescents of 0.99 gm, and the 8 puppies of 0.69 gm.

Although only 86 parathyroid glands, loose in the capsule or imbedded in the surface of thyroid lobes, were weighed, 119 glands were found and the presence of each was confirmed microscopically. The additional 33 were so firmly imbedded in the substance of the thyroid lobes that weighing was both impractical and inaccurate. These imbedded glands were found by making slices 0.5 mm thick across each lobe of the thyroid gland and were so obviously small (less than 0.5 mg) that no significant alteration of the weights of the thyroid lobes containing them resulted. Of these 119 parathyroid glands, two were found in 5 dogs, three in 11, and 4 in 19. Of the 60 superior parathyroid glands identified, 51 were located over the cranial half of a thyroid lobe free in the soft tissue at, anterior or posterior to, lateral to, or medial to the upper pole of the lobe. Of the 9 remaining superior parathyroid glands, 8 were imbedded in the cranial half of a thyroid lobe and one was free over the lateral surface of the caudal half of a thyroid lobe. Of the 59 inferior parathyroid glands identified, 25 were free in the soft tissue or adherent to the surface of the caudal half of a thyroid lobe in anterior, lateral, posterior, or medial positions. Twenty inferior parathyroid glands were imbedded in the caudal half of a thyroid lobe, frequently along the posterior margin and 14 were either free in the soft tissue or imbedded in the substance of the cranial half of a thyroid lobe. In two dogs, one superior gland was split into two closely related parts and in one dog, an inferior gland was so affected. In two dogs, one extra parathyroid gland was found within the substance of a thyroid lobe. In only two dogs, small nodules of accessory thyroid tissue were found, two in one dog and one in the other.

Of the 86 parathyroid glands weighed (1 in 2 dogs, 2 in 20, 3 in 8, and 4 in 5), 66 were 1 to 5 mg, 10 were smaller than 1 mg, and 10 were larger than 5 mg. The weight of the parathyroid glands ranged between 0.1 mg and 6.6 mg with a mean of 2.7 mg (fig. 2). The sigma was 1.8, the sum of the weights of the 86 glands was 233.8, and the sum of the squares of the weights was 904.64. By dividing the glands among the three age groups indicated for the thyroid gland, the 35 glands from adults had an average weight of 2.8 mg; the 26 from adolescents, 3.0 mg; and the 25 from puppies, 2.4 mg.

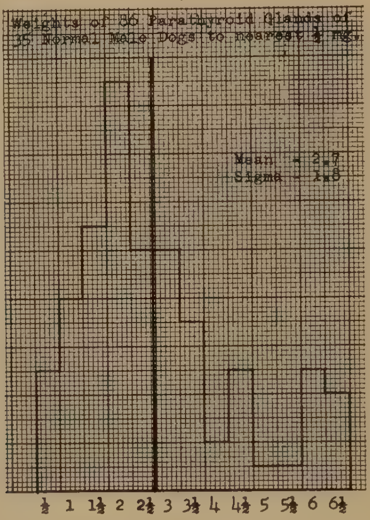


Figure 2

In figure 3 is depicted the relationship between the weight of the thyroid gland of 34 dogs and their body weight. The weight of the thyroid gland of the remaining animal, 2.03 gm, was not included, since the graph would have had to be extended impractically long on the y-axis to plot only one point, which would not have influenced the slope of the curve. A direct correlation between the weight of the thyroid gland and body weight is evident. A similar graph failed to prove any correlation between the weight of the parathyroid glands and body weight.

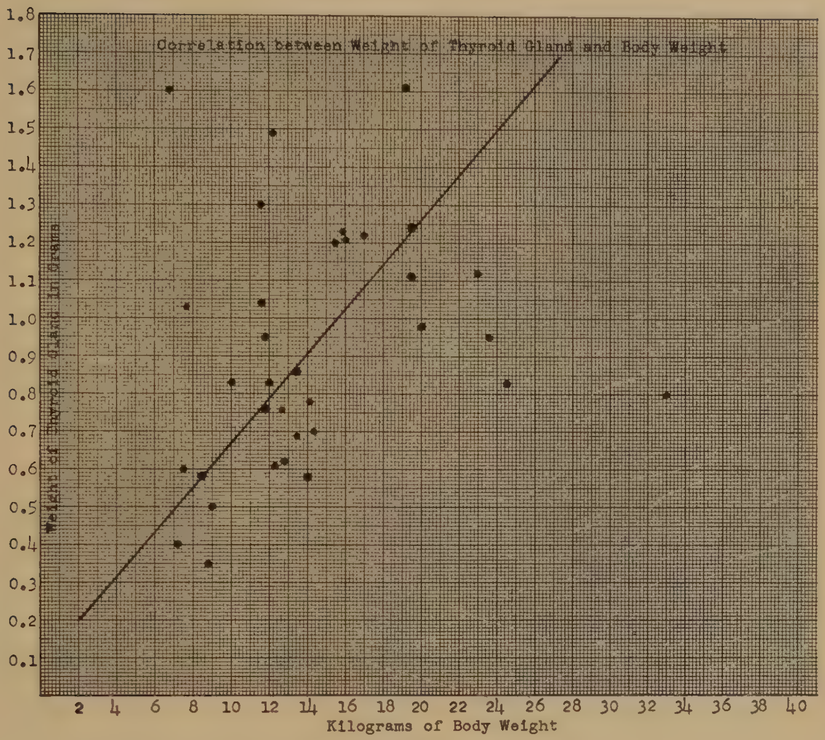


Figure 3

SUMMARY

1. The thyroid glands of 35 normal male dogs ranged in weight between 0.35 and 2.03 gm with a mean of 0.95 gm and a sigma of 0.37.

2. The 86 parathyroid glands of 35 normal male dogs ranged in weight between 0.1 and 6.6 mg with a mean of 2.7 mg and a sigma of 1.8.

3. A direct correlation between the weight of the thyroid gland and the age and body weight of the dogs was observed. No relationship was found between the weight of the parathyroid glands and the age and body weight.

LITERATURE CITED

Francis, K. C., and R. M. Mulligan 1949 The weight of the pituitary gland of the male dog in relation to body weight and age, with a differential cell count of the anterior lobe. J. Morph., *85:* 141–161.

ASPECTS OF NEURITE OUTGROWTH IN TISSUE CULTURE[1]

C. E. LUMSDEN[2]
WITH THE TECHNICAL ASSISTANCE OF
MARY F. ORR AND DOROTHY ROBBINS
AND PHOTOGRAPHY BY
CURTIS HOSKINS AND GEORGE LEFEBER
Tissue Culture Laboratory, University of Texas, Medical Branch, Galveston

TEN FIGURES

The study of the living nerve cell and its processes is the oldest subject in tissue culture; indeed the tissue culture discipline as a method in experimental biology dates from the original studies made by Harrison in 1907 on the behaviour of nervous tissue from frog embryos grown in frog lymph. There is by now a vast literature on the behaviour of nervous tissue *in vitro* in the case of the embryos of avian, amphibian and mammalian species, and interest more recently has turned to the unexpectedly rewarding field of adult human nervous tissue where most interesting observations have already been published by Murray and Stout ('47) on adult human sympathetic ganglion cells cultivated outside the body, and by Pomerat, Ewalt, Snodgrass and Orr ('50) on cells cultivated *in vitro* from fragments of adult human cerebral cortex.

As far as the neurone itself is concerned, "outgrowth" *in vitro* in the case of explants of embryonic nervous tissue commonly used (fragments of brain, cord, optic vesicle, and sensory ganglia) has been up to now uniformly restricted to the outwandering of neuritic processes only; while the nerve cell nucleus, the perikaryon, has consistently revealed itself as singularly incapable of migration and multiplication. However, the observations of Murray and Stout on adult human sympathetic ganglion cells, and possibly also those of Pomerat on adult human cortical ganglion cells (personal communication) indicate that this generalization may not apply for neurones transplanted to culture from the adult organism. It may therefore be that the incapacity of embryonic perikaryon to migrate in culture may have some explanation other than the alleged inherent unsuitability of the culture milieu. This fascinating problem, however, is of collateral interest only to the present paper.

---

[1] Aided by a grant from the U. S. Public Health Service, RG 333 (C2), administered by C. M. Pomerat.

[2] Rockefeller Research Fellow. Author's home address: Dept. of Pathology, University of Aberdeen, Scotland.

In tissue culture, the relative incapacity of the nerve cell to migrate and to multiply is compensated for by the greater measure of differentiation, and to some extent "organization," which it displays in conformity with the general law (Strangeways, '24; Fischer, '46; Weiss, '49; and others) that, in tissue culture, differentiation and proliferation are antagonistic. How remarkable indeed this tendency is, it is one of the purposes of this paper to reassert. This measure of differentiation and organization, peculiar to the neurone *in vitro,* has been fully appreciated by all workers in the tissue culture field but many experienced workers like Levi ('41) have preferred to minimize its significance on the grounds that it is a transitory phenomenon. The specificity of this differentiation and organization is so marked and so constant, however, that it merits more emphasis; and less stress should be laid on its transitoriness which, after all, merely reflects the unsuitability of the milieu to sustain the subsequent phases of development of a nervous system *in vitro.*

It cannot be emphasized too strongly at the outset that the few cultures used in this report do not permit anything like a complete description of neurite morphology in tissue cultures of posterior spinal root ganglion from chick embryo. This has been done supremely well already in the long and excellently illustrated paper by Levi ('41). Indeed it would be a presumption on the part of the present writer to seek to add anything in the morphological descriptive field to this thorough research by Levi who was a pioneer in the *in vitro* study of the neurone from 1917, and who has continued to pursue his investigations actively since then. Preparations from intact posterior spinal root ganglia yield neuroblasts of less pleomorphic character than sectioned ganglia (Levi, '41) and the minimal pleomorphism and the minimal reactionary characters of the present preparations have provided a relatively simplified morphological system here in which it has been our aim to illustrate the essential features of neurite differentiation and organization in tissue culture and to attempt to review the factors which may be responsible for these phenomena.

METHOD

The explants consisted of whole dorsal spinal root ganglia of a chick embryo in its 9th day of incubation. They were grown in the standard way in plasma clots made by mixing equal amounts of rooster plasma and chick embryo extract on cover slips, and these were then maintained simply as "hanging-drop" preparations.

After one week's incubation at 37.0°C. they were fixed in formol bromide and stained by Bodian's protargol method.

DESCRIPTION

(a) General. The preparations were those of a typical viable explant of chick embryo dorsal root ganglion showing an abundant outgrowth of neuritic processes extending for 2 to 3 mm beyond the margin of the explant. Typical, too, was the luxuriant outgrowth of undifferentiated mesenchymal cells, and, in smaller proportion, of cells of Schwannian character. No nerve cell bodies were found anywhere in the outgrowth but these cells formed the most conspicuous element within the explant itself. Most of the preparations, particularly that used for the present series of illustrations,

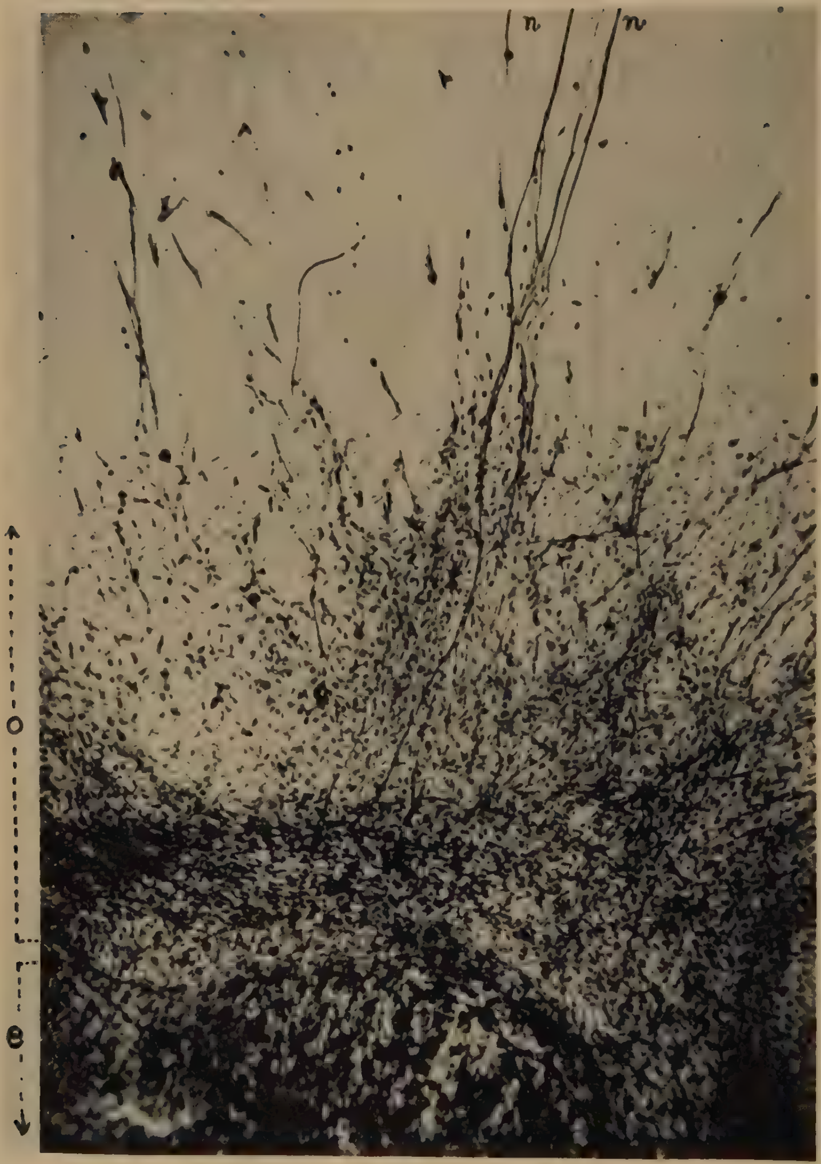


Fig. 1 Explant of 9-day chick embryo posterior spinal root ganglion after one week's growth in hanging drop culture. Formalin-fixed; stained by Bodian's protargol method; × 47. ''e,'' explant; ''o,'' zone of mesenchymal and neuritic outgrowth; ''n,'' neurites.

had thinned out well in the course of the week's culture, as is commonly the case, and most regions could readily be examined even with an oil-immersion objective.

Within the explants the large cell bodies of the neurones lie scattered in weaving meshes of neuritic processes on a background of undifferentiated rather small cells (fig. 3) similar in appearance to those in the outgrowth where they have been identified as mesenchymal and Schwannian cells. The neuronal bodies show well marked neurofibrillary structure, and throughout the explant they show as yet few or no degenerative stigmata unless, following Levi and Meyer ('45), the slight corrugation of the neurofibrils within the perikaryon seen in some of them be taken as evidence of early damage to the cells.[3] For the most part the neurofibrils within the cell bodies show what appears to be a normal state of anastomosis and interlacing; followed out into the axonic processes they become parallel and remain discrete from each other.

Notably, throughout the present few cultures, the neurones are cells with solitary, well-developed, axone-like processes. Relatively few bipolar and no multipolar forms have been encountered. The cell bodies are fairly uniform in size, averaging about 20 μ in diameter, and the giant bizarre forms described by Levi in older cultures by the hanging-drop method are absent.

(b) Detailed description of neuroblast illustrated in figure 2. In the course of study of the microscopic preparations the interesting neuritic termination shown in figure 8 was seen. Followed back to its origin it was found to arise from the cell body shown in figure 3. This continuity was confirmed in several detailed studies of the preparation. Because of the thickness of the explant and of the plasma clot and because the nerve process undulates in depth as well as laterally, it was not possible to photograph the whole neurone in one exposure. Recourse was therefore had to multiple

---

[3] This is the sort of neurofibrillary change which Murray says ('47) recovers in culture in about one month.

serial exposures bringing each segment of the fibre into sharp focus under oil-immersion objective. Since great care was taken to include a wide margin of overlap in each photograph those features common to adjacent photomicrographs (characteristic loops of fibres and contours of nuclei, nucleoli, etc.) were readily identified. The prints were then cut down with scrupulous care to restore the true continuity of the fibre and its neighbouring structures. These 35 prints were then pasted together on art board to produce the reconstruction shown in figure 2. Needless to say, an identical magnification (2000 times exactly in the original prints) and identical optical conditions were used throughout.

Located in the vicinity of the termination of the complete neurone shown in figure 2 were the two axonic terminations shown in the inserts to figure 2 and reproduced in full scale in figure 9 and 10. Unfortunately the neurites bearing these interesting terminations could not be traced back with certainty beyond the margin of the explant where they became involved in a tangled mass of fibres and cells too thick for the somewhat delicate procedure of tracing fine undulating fibres.

Some measurements from the reconstruction are of interest and are probably more accurate for the purpose than could be obtained directly by a micrometer eyepiece. At this magnification of × 2000, the diameter of the cell body (fig. 3) is 4.32 cm. The length of the process, measured with a planimeter, is 259.1 cm; that is to say, the axonic process is 60 times the diameter of the parent cell body. Given that the magnification is exactly × 2000, the true length of the axonic process is therefore 1.3 mm. As can be readily confirmed from figure 2, just over two-thirds of the axone is in the outgrowth, i.e., approximately 1 mm.

Inspection of figure 2 shows that the course of the fibre is by no means a straight radial one. Within the explant it executes several obtuse-angled bends after leaving the pyrenophore, then near the margin of the explant it performs a turn of about 90° in one direction and then a sharp turn of about 75° in the opposite direction just before it leaves the outer limit of the margin of the explant. Thereafter, in the outgrowth, it pursues an undulating course for about 500 μ in a general direction of about 45° to the tangent to the circumference of the explant made at the point of exit of the fibre. Then it swings outwards through a wide angle of about 125° back into a direction roughly parallel to its original course through the explant. Just before its termination is another slight change of course of about 45°.

Other fibres pursue similar angular courses. Within the explant such angulation can be very sharp indeed as figure 4 from a neighbouring fibre shows. Notably absent in these preparations is the tendency to circumferential growth by the outgrowing neurites immediately around the outside of the explant noted by Levi ('41) in some of his preparations.

Perikaryon. Figure 3 shows the nerve cell body with laterally placed nucleus (a transitory phase in development *in vitro* according to Levi) and the neurofibrillary structure to which reference has already been made. Careful focussing revealed only a single process arising from the cell — the axone "a" — in which neurofibrillae can be clearly seen. Throughout the explant the perikaryon of other neuroblasts show very similar appearances. The slightly corrugated structure of the neurofibrillary apparatus which has been accepted, following Levi and Meyer ('45) as a recent degenerative change, is in fact seen in only a few of the cells. The cell bodies here are remarkably uniform in size. That shown in figure 3 measured 21.6 μ in diameter which corresponds, assuming it to be nearly spherical, to a volume of about 5000 μ^3. In Levi's material the volumes varied from about 2000 μ^3 to 124,000 μ^3, the last being larger than anything encountered in the adult hen.

Axones. Within the explant the nerve fibres, which, as figure 3 shows, are profuse, weave and turn in all directions crossing each other, converging and diverging apparently indiscriminately. But in the outgrowth a tendency to bundling or fasciculation becomes clearly displayed, as seen in figure 2. Sometimes the bundle is loosely packed and individual fibres undulate from side to side while one or two, for no visible reason of mechanical obstruction, swing away from the main stream (figs. 2 and 5) out into the surrounding mesenchymal syncytium usually to join eventually some other bundle of processes often at some considerable distance away. Sometimes such a fibre again leaves the second bundle and returns to its original path. Whole fasciculi, frequently from a considerable distance away, converge like streams of railroad tracks towards a junctional point; and may diverge and reconverge forming a plexus-like arrangement. Here and there the fasciculi become very tightly packed so that it is extremely difficult to trace any individual fibre (fig. 6) and the whole structure presents the appearance of a compact little nerve bundle. No mechanical reason, such as a dense accumulation of cells in the vicinity nor any obvious fault or aberration in the plasma clot can be detected in the stained preparation (where ''ground mat'' stains) in fields like figure 6 to explain this tight bundling. Though in such regions as in figures 5 and 6 individual fibres may run closely apposed for a considerable distance, careful focussing shows that they remain discrete. It has been found impossible in any instance to demonstrate evidence of actual fusion of the axoplasm from adjacent fibres.

In the outgrowth, the surrounding cells are poorly differentiated and form the coarse syncytium shown in figures 5 and 6. Two types of nuclei, however, may be fairly clearly distinguished: (1) the majority being pale, large, rounded or kidney-shaped, i.e., those considered to be undifferentiated mesenchymal cells; (2) smaller, darker, elongated nuclei accompanying the nerve fasciculi and presumptively of Schwannian type (fig. 7). Throughout the outgrowth, numerous mitoses are seen amongst both types of nuclei. The mitotic figure shown in figure 7 is presumably a dividing mesenchymal cell whereas that shown in figure 8 is, partly in view of its elongated character but even more so in view of its integral relationship to the terminal nerve filaments, evidently Schwannian. It would appear, indeed, in this latter instance, that these terminal nerve filaments had already become invested in some sort of fine sheath enclosing not only these filaments but the Schwannian cell too, and that the great increase in volume of the cell during its division is responsible for ballooning the investing sheath and so causing the curious perinuclear loop taken by the nerve filaments.

Nerve endings. Figures 8 a, b and c, are taken from the same field but each at slightly different focus in order to show fine detail of the end loops and rings on these terminal nerve filaments. About half of the processes in the outgrowth terminate in such rings or loops, or occasionally (fig. 9) in more solid "boutons terminaux." Others terminate by breaking up into very fine arborizations; these terminal twigs are usually distributed freely amongst the syncytial cells. Since no clear cytoplasmic membranes have yet formed here it is not possible to speak of *penetration* of the mesenchymal cells by the terminal filaments such as Grigorieff ('31, '32–'33) has described. What is, however, very striking is that the terminal arborizations of two nerve fibres sometimes come to lie in a curious quasi-synaptic relationship to each other (fig. 10).

DISCUSSION

(a) The neural pattern in vitro in some respects constitutes a nervous system in miniature

Ontogenetically, three stages of the neurone are recognized (Ariens Kappers, '32). In the first stage no offshoots whatever are seen; in the second stage the axone appears; and, in the last stage, dendrites grow out. In the present cultures from posterior spinal root ganglia of an 8–9 day chick embryo the neuroblasts show well-developed axones but no dendrites. This, indeed, is the exact picture which Harrison originally described ('07) in the case of amphibian neuroblasts growing in frog lymph. In this restricted sample of material the marked pleomorphism which Levi ('41) obtained with cut samples of sensory ganglia from chick embryos is absent. Only a small proportion of neurones in the present material are bipolar and no multipolar forms have been seen; nor any of the giant bizarre forms found by Levi in old cultures (after 37 days) in Carrel flasks. Levi was able to show how the age of the tissue explanted, the time in culture, the temperature of incubation, particularly the method of culture used, and actinic emanations can all produce relatively enormous changes or modulations in the morphology of cultures. He was careful, however, to say that such factors cannot explain certain characteristics of neurite outgrowth. In Levi's material the majority of these pleomorphic changes were not spontaneous but were induced by conditions which were evidently artificial for the tissue such as excessively prolonged incubation, an incubation temperature abnormal for chick embryo, radioactive emanations, traumatized ganglia and so forth. Nevertheless, in most of his cultures, there seem to have been many monopolar neurones, without branching or collaterals, as in his figure L, p. 168 (Levi, '41).

These second-stage neuroblasts resemble the simple primitive neurosensory cells which exist apparently throughout the whole phylogenetic system from Coelenterates upward (Ariens Kappers, '29). Compared with these nearly universal primitive neurosensory cells, the second-stage neuroblasts in the present cultures are remarkably evolved morphologically. They have a well-formed neurofibrillar system, and long axones of which by no means the longest example has been shown here to be 60 times the length of the parent cell's diameter; they have terminal filaments, rings and loops, and possibly even rudimentary synaptic connections with each other. With regard to these last, which in fact most authors have observed (Levi, '19; Grigorieff, '31–'33), Levi warns that interpretation must be particularly guarded as, in his view ('41), such arrangements may be transitory. To insist that the term *synapse* must be reserved, *in vitro* as *in vivo*, for a durable structure in connection with a receptor cell[4] is undoubtedly a fair judgement. However, even if such quasi-synaptic relationships are transitory, their formation may yet not be entirely without significance when it is borne in mind that it is only in a very short transitory phase of the tissue's growth *in vitro* that the culture milieu is suitable for its normal development.

It seems to the writer that, all considered, the neurone in culture shows a remarkable degree of evolution in form. On the basis of morphology alone there would seem to be no fundamental reason why such neurones should be incapable of normal neural activity. Indeed, it is possible that the development of neurofibrillae may indicate that currents of electrical conduction may have already commenced within the neuroplasm. Neurofibrillae are not themselves the conduction pathways (Parker, '29) but they are probably the product of conduction (Ariens Kappers, '32). As Ariens Kappers says, analagous fibrils may also arise in other cells than nerve cells if they are frequently passed by an impulse current. Thus, in addition to the morphological completeness of these second-stage chick neuroblasts we have to credit them likewise with some measure of functional activity. The only further evolutional change these cells have yet to achieve in progress to the adult form is to acquire dendrites. It might well be observed that dendrites are probably necessary, ontogenetically as well as phylogenetically, *not* for releasing the processes of electrical conductivity but merely for enriching the possibilities of association between neurones and for providing the fundamental possibility of choice or selection of the pathways of conduction through the excitable nervous system.

---

[4] ''Pour réaliser une synapse *in vitro,* il faudrait que les branches du neurite d'un neurone sensitif contractent des relations matérielles durables avec une cellule réceptrice; cette condition n'est jamais produite dans mes cultures, et, selon mon opinion les résultats positifs en ce sens que quelques auteurs prétendent avoir obtenus, ne sont pas suffisamment probants.'' (Levi, 41, p. 173).

With regard to the axones and the terminals in the present preparations some other morphological features are worthy of comment. It is notable, for instance, there is no evidence of mergence of the axones however closely apposed they run. Even with all his preparations representing such a varying range of conditions, this was likewise the experience of Levi ('41). Compared with other migrating cells whether of epithelial or mesenchymal origin, or even for instance with oligodendrocytes in culture (Lumsden and Pomerat, '51), this discreteness on the part of the neurites is remarkable. It has been noted, too, that whereas the nerve fibres within the explants run in all directions, apparently indiscriminately, in the outgrowth they appear to follow prescribed courses. What the forces responsible for this grouping and directionation of the fibres in the outgrowth are will be discussed presently. Meanwhile, it cannot be gainsaid that there is at least a superficial resemblance between the nerve pattern of the neuritic outgrowth *in vitro* and the neuroanatomical pattern of "organized" tissue *in vivo*. The aggregation of the fibres into bundles, which are virtually slender nerves, has already been described. Such bundles are surely reminiscent of the terminal fascicles which supply individual muscle fibres, which emerge from complex sensory apparati, or which innervate the serous membranes; and some of the features here illustrated from the tissue culture outgrowth might equally well be found in the illustrations, for instance, in Boeke's section in "Cytology and Cellular Pathology of the Nervous System" (Penfield, '32). Even the plexus-like interconnections between individual bundles which occur in the outgrowth *in vitro* simulate those seen, for instance, in Auerbach's plexus of the mammalian small intestine. Likewise, nerve terminations described here have their counterparts in the neural apparatus of adult mammalian tissue: the free arborizations seen in figure 10 might be an illustration of those in the human peritoneum; the terminal rings and loops of figure 8 resemble the "terminaison en grappe" structure of motor end plates to skeletal muscle as found in vertebrates from Reptilia upwards (Boeke, '32). Indeed, the formation of such structures in tissue culture has already been described by Lewis ('24), by Levi ('26) and by Mossa ('29). It is extremely interesting, then, that this simple "nervous system" *in vitro* is potentially motor or sensory — unless we take the incredible standpoint that the outgrowing neuroblasts here are efferents connected in some way with the scanty efferent vasomotor elements which are thought may run *via* the dorsal roots (Fulton, '43). It is tempting to speculate in conformity with the view outlined in the subsequent section of this discussion that, until dendrites have grown out from the parent neurone, the axone behaves in accordance with its inherent capacity of conducting an impulse in either direction; only when morphological bipolarity is established does afference or efference become specifically manifested. We shall return to these fascinating speculations later in reference to the recent important observations of Murray and Stout ('47) on the polarity of migrating nerve cell bodies in cultures of adult human sympathetic ganglia.

(b) Factors regulating neurite outgrowth

The complex problems concerned in the migration of cells in tissue culture have been discussed clearly and in great detail in Fischer's recent monograph "Biology of Tissue Cells" ('46). Aspects of the same problems pertaining more especially to protoplasmic movements ultimately responsible for morphological differentiation have also been reviewed recently by Weiss in an essay on "Differential Growth" which appeared in the Princeton University symposium entitled "The Chemistry and Physiology of Growth" ('49).

In view of the complexity of the problems which these two reviews have shown to be concerned, it is perhaps oversimplifying and artificial to attempt to list the various factors which have been credited by different authors with the essential determinizing role in cell migration and differential growth. It is evident that living protoplasm is "directed" ultimately at the molecular level where energy is freely negotiable in physical, chemical or electrical currencies.

Generally, however, there have been those on the one hand who have attributed the chief directional forces in cell migration and in the movements of protoplasmic processes to more purely chemical factors such as a radial concentration gradient of metabolic products, or, more specifically, to a differential oxygen-carbon dioxide gradient between the explant and the surrounding medium. This is "*chemotropism;*" and its reasoning, as Fischer succinctly states it, is as follows: "The migration will continue until the cell is in equilibrium with the medium, which means up to the time when cells no longer are exposed to influences from a one-sided diffusion field."

On the other hand, there have been those who have emphasized the predominant role of physical factors such as *stereotropism,* or *thigmotropism.* In this field, Weiss's investigations have been particularly important. He has emphasized the role of mechanical strains and tensions within the highly elastic plasma clot which is the essential substrate *in vitro* for migration and differentiation of cells. He has demonstrated in a long series of experiments that tension has a "spectacular . . . effect on the architecture (of tissue) not only of mesenchymal origin but also on epithelial tissues in culture." "Connective tissue, tendon, fascia, cartilage, bone, blood vessels, and muscles all respond to mechanical tensions by orientation of the component cells and intercellular fiber systems along the lines of stress. Experimental analysis of this response in tissue culture showed that the observed cell orientation is a secondary product following a primary orientation of the colloidal matrix in which the cells lie embedded" (Weiss, '49). The lines of tension within the matrix of plasma clot produce alignment of the long-shaped molecules into chains, these into submicroscopic fibrils and these last into microscopic fibres. Cytoplasmic filaments from the growing cells extend, by capillary or thigmotropic effect, along the interfaces between these solid micellar threads of this protein "ground mat" (Weiss, '45) and the interstitial fluid. The cell body follows. "In this manner the cell becomes greatly elongated and is made to trace the ultrastructural pattern of its surrounding" (Weiss, '49). And, what is particularly significant to the present study, he concludes also that "nerve processes advance in the same fashion except that their cell body remains anchored." In this principle, to which he has referred variously in his papers as "the principle of contact guidance" ('41), "interfacial tension," and "the concept of the oriented ground mat" ('49), Weiss has sought to offer a "realistic explanation . . . for many of the morphogenetic processes formerly ascribed to various ill-defined cell 'tropisms' " (Weiss, '49).

At first consideration this explanation by Weiss would seem to imply that the direction of the outgrowing neurite is under the control of factors entirely extrinsic of the neurone. If this were the case it would be difficult to understand why migrating structures like epithelial cells, mesenchymal cells, vascular endothelium and neurites behave in a visibly different way towards the same substrate; in other words, why cells in culture do not "dedifferentiate" morphologically more than they do. Weiss explains that cells in culture undoubtedly retain their biochemical identity, or "differentiation" in this sense, regardless of any modulation in morphology. Different cell types will therefore continue to elaborate "different lytic discharges" (Weiss, '49) such as specific proteolytic and lipolytic enzymes, and that these will coat the various contact surfaces over which the cells and processes are guided. In this way, if the writer has interpreted Weiss correctly, the intrinsic nature of the growing cell while not determining the actual pathway which a cell or a process will follow determines the surface tension and viscosity of the milieu in its immediate environment. In this way the modulation of the process and its rate of advance are partly determined by the nature of the cell itself. Indeed, as far as the actual form of the cell body itself is concerned, Weiss is careful to say that extrinsic forces are not determining factors — "Stress is decidedly not a determining factor in cytodifferentiation. Though it seems to be prerequisite for some cell types in order that they may be able to carry out terminal transformations for which they are already predisposed by previous differentiation, it is not the cause of such differentiation. Without stretch, myoblasts cannot transform into muscle fibers but stretch itself neither makes a cell into a myoblast nor ever transforms a nonmyoblast into a muscle fiber" (Weiss, '49, pp. 176–177).

In the specific instance of the neurite outgrowth which we have followed in detail throughout its course, a study of the photomicrographs makes it seem probable that mechanical stress factors are responsible for the frequent irregular minor undulations of the courses of individual fibres, and for the angular turns, acute and obtuse alike, occasionally encountered. Whether they are responsible, too, for the large swinging deviations of course illustrated it seems impossible at the moment to assert. But the more specifically "neural" features of the outgrowth which mimic a miniature nervous system — i.e. the fasciculation, the plexus formation, the orientation of nerve terminals in relation to each other, and even the specific affinity of the Schwannian cells for the neurite — seem to imply control by forces within the neuronal protoplasm. It is precisely these features of characteristic orientation of neurites within the outgrowth, of fasciculation, and of plexus formation, for which others like Levi ('41, pp. 233–243) who, though seeking it, have been unable to find any satisfactory explanation in terms of physical factors extrinsic to the neurone. Levi, for example, finds that in the outgrowth it is the very slender neurites which are deviated; the robust fibres are able to continue a straight course peripherally. In the same way, Olivo ('27a, b) was convinced that chemical and mechanical factors cannot alone be responsible for directing the nerve cell axis: experimentally, he showed that in explants from brain, neurites migrating fanwise from multicentric foci of outgrowth would cross each other's trajectories readily without the fibres from one focus becoming deviated from their original direction to follow trails already taken by fibres from another focus.

In addition to making a reservation to his stress theory in respect of cytodifferentiation, Weiss has pointed out that tension is not the only external physical force that is capable of affecting the orientation and aggregration of polar molecules (Weiss, '49), but that electrostatic fields, electrophoresis, and streaming, may have comparable effects. Presumably he is still thinking, as in his study of 1934, of the possible role of these extrinsic electrical forces orientating the polar molecules of the "ground mat" and thereby directing the movement of the neurite rather than of electrical forces conducted within the neurite; for to admit the latter would be to admit something which if not a neurotropism must be something closely akin to it — and "neurotropism" is a force the existence of which Weiss specifically refutes (Weiss, '44).[5]

That externally applied galvanic currents can in fact control the direction of outgrowing nerve fibres in tissue culture was first reported by Ingvar in 1920. This finding was supported by the experimental evidence likewise of Péterfi and Williams ('33 and '34) and of Karssen and Sager ('34) but was not confirmed by Weiss ('34), Levi ('34) and Williams ('36). It was, however, conclusively substantiated by the experiments of Marsh and Beams ('46) who showed that the failure of earlier workers who had failed to demonstrate control of growth of nerve fibres by electrical currents were due to their use of current strengths too small to produce visible effects. Indeed Ingvar himself may have underestimated the currents employed in his own experiment for, in order to obtain their positive results, Marsh and Beams found it necessary to use currents 50,000 times as strong as Ingvar stated his to be. However, high as were the potential gradients employed by Marsh and Beams at the orientation threshold compared with those given in Ingvar's paper, they are still small when compared to the gradient produced by the rheobasic voltage for direct current stimulation of frog nerve and "conspicuously less than the gradients produced in many bioelectric systems as frog skin, electric organs, protoplasm of Nitella, etc., and may be considered as physiologically attainable" (Marsh and Beams, '46, p. 155).

---

[5] I have used the term "neurotropism" in a strict sense to imply orientating influences or affinities within and between specifically neural elements, i.e. to imply a specific property of neuroplasm. The term has, of course, been used in a more vague sense to imply a hypothetical attraction exerted upon outgrowing neurites by other cells like mesenchyme and epithelial cells (Grigorieff, '31, '32–'33) or muscle cells (Péterfi and Williams, '34b) in cultures. The writer has no reasons here to infer that such forces exist *in vitro*.

One aspect of Ingvar's experiment which does not seem to have been followed up by later workers is of especial interest here. He stated that when the weak electrical current employed in his tests was drawn through the centre of a culture by means of a single conductor, the outgrowth of fibres and cells always occurred perpendicular to the conductor. In this latter finding lay the experimental counterpart of Bok's observation on the developing central nervous system of the chick embryo *in vivo.* Bok showed that the first axones to appear in the nervous system are not root fibres but the neurites of arcuate fibres forming the posterior longitudinal bundle which develops first from cells of origin in the midbrain and grows caudad into the spinal cord; when, a little later, the neuroblasts of the cranial motor roots appear they become orientated perpendicularly to this bundle with the axones turned away from this tract apparently in response to some influence emanating from the tract. Ingvar, and Ariens Kappers, saw in this a galvanotropic phenomenon too; and Kappers saw in it an instance of a general morphogenetic principle of first importance in the nervous system, the principle of *neurobiotaxis* (Ariens Kappers, '21, '29, '32).

From the point of view of the present discussion, it must be conceded that experiments with galvanic currents applied to tissue cultures do no more than show, so far, that a galvanic current can control the direction of an outgrowing neurite; they do not yet show whether the directing impulses are carried intrinsically within the neurite or whether they act extrinsically merely by orientation of the molecules within the "ground mat," as Weiss inferred (Weiss, '34). Evidence from two different sources, however, suggests that the orientating effect of the galvanic current is intrinsic within the neurite and does not act through the ground mat. Firstly, there is the fact that different types of migrating cells in cultures respond differently to electrical currents. Peterfi and Williams ('33, '34a and b), for instance, carried out extensive experiments on the effects of galvanic currents applied to different types of cells in tissue culture and elucidated their differential behaviour. Among those cells manifesting no response whatever were epithelial and mesenchymal cells, large monocytes and sarcoma cells. The only cells showing a marked structural response were neuroblasts, with typical polarity. Certain cells like small monocytes, leucocytes and microgliocytes showed minor cytoplasmic movements with some tendency to polarity but no permanent structural alteration like the neuroblasts. Their results would therefore seem to indicate that the structural alterations caused specifically to neuroblasts by electrical stimulation must have operated intrinsically upon the neuronal protoplasm rather than upon the ground mat. Secondly, Marsh and Beams ('46) found that the orientating effects of galvanic currents were the same whether in 18 hour old plasma clots or in freshly made preparations, whether in dense or in "nearly fluid" clots. Even more cogently, they showed that when, in the same preparation, the direction of the applied current was changed in the course of its exposure, orientation of the continued growth occurred towards the new cathode. On these grounds Marsh and Beams considered it "unlikely that the current produces micellar orientation for it would be necessary to assume that the micellae reorient when the current direction is changed" (Marsh and Beams, '46, p. 154).

Let us turn from the artificial conditions involved when galvanic currents are applied externally to the natural conditions of the undisturbed growing culture. Here the experimental proof of "neurotropism," in the sense of spontaneous intraneuronal galvanotropism, would entail the demonstration in tissue culture of naturally occurring electrical conduction within the nerve fibres. Failing experimental demonstration of this so far, recourse must be had to indirect evidence. This is by no means lacking, and the following arguments may be listed:

First, it is known that spontaneous electrical currents flow in developing organisms in the normal course of their development, so that embryonic tissues are by no means electrically inert (Ariens Kappers, '32).

Second, as Harrison himself showed ('07), when portions of myotomes are left attached to a piece of medullary cord the muscle fibres which develop well after two or three days exhibit frequent contractions. Without this neural connection, contraction does not occur. This would seem to indicate, then, that action currents do occur spontaneously in tissue cultures, at least intermittently.

Third, the recent studies already referred to, by Murray and Stout, upon the behaviour of the adult human sympathetic ganglion cell, show that the neurone retains even *in vitro* its fundamental property of polarity once this has been established in the tissue from which the explant is taken. If the abnormal nature of the environment within a culture chamber does not destroy polarity in the adult nerve cells there is no reason at present to see why it should do so in the embryonic nerve cell if it were already established in the embryo before explantation of the ganglion. There is this curious morphological difference that whereas the pyrenophore of the embryonic neuroblast remains stationary within the explant while its axone is thrust out actively into and through the outgrowth, in the adult neuroblast the growth polarity is to a large measure reversed and the axone remains relatively fixed while the dendrites and cell body migrate outwards. To this can be found a truly remarkable parallelism in the response of neuroblasts in culture to electrical stimulation; for, according to Péterfi and Williams ('34a; pp. 231–

235) whereas young neuroblasts were anodotropic, aging neuroblasts became cathodotropic.

Fourth, it has already been pointed out in part (a) of this Discussion that it is possible that the existence of neurofibrillae themselves in perikarya and axones may indicate that electrical conductivity has already been established.

The really fundamental quality of differentiation of the neurone *in vitro* must lie as *in vivo* in the biochemical specificity of the neuroplasm which endows the cell with its characteristic irritability. Since the neuroblast of the embryonic nerve tissue and the adult neurone itself both manifest in tissue culture a degree of morphological differentiation which is indeed unique for all tissues *in vitro* then it would seem a logical assumption that, biochemically, the neurone is no less advanced in differentiation that it is in structure in these respective instances. If the problem is viewed in this way, therefore, it would seem not at all improbable that even the neuroblast in the explant of the posterior spinal root ganglion of the 9-day chick embryo is already an electrically functional unit. It is true that this neuroblast is not as yet part of a complex system of communication and not yet called upon to interpret and transmit stimuli received from without, unless externally applied by artificial means, but the whole molecular machinery is already there, meanwhile transmitting the intracellular currents that must surely arise in living protoplasm destined eventually to express itself by this particular property of electrical excitability. Following the example of ontogenetic evolution, throughout the period of its development and growth there must be a period of tentative function which, because limited in power is as yet ineffective for the final purposes of intercellular communcation, would still be used for some simple purposes ancillary to growth. It is this concept which Ariens Kappers ('32) has generalized in the dictum: "Function precedes Form." By this line of reasoning, its neuroelectrical potentialities will be utilized for the purposes of growth of the neurone and its processes. The perikaryon, while synthesizing molecules for the ever growing axone — as, it is known, it continues to do even in the adult organism — may likewise supply small and intermittent action currents sufficient to shape the course of the advancing tip of the fibre. This latter in its outward progress undoubtedly accepts from the environment, which it is destined ultimately to serve, those mechanical and chemical stimuli which guide it to a position of final equilibrium; but, throughout its whole existence, the dominant force it obeys is the impulse, the action current, it receives from the parent cell body. And, as a corollary, those extrinsic forces which are likely to affect its outward course most, whether *in vitro* in experiments of Ingvar's type, or *in vivo* in neurobiotatic development, are the electrical forces which of all forces are most readily accepted and interpreted by the specialized bioelectric mechanisms of nerve protoplasm.

SUMMARY

An attempt has been made to illustrate the essential features of neurite differentiation and organization in tissue culture, and to review the factors which may be responsible for these phenomena.

It is concluded that the chief orientating factors in neurite outgrowth in culture are intrinsic within the neurone and that physical stress factors operating in the "ground mat" of the plasma clot play only a subsidiary role.

It is considered probable that even in culture, as in the intact embryo, developing neuroblasts are capable of electrical activity and that this may be in some measure responsible for directing the outgrowing axone.

LITERATURE CITED

Ariens Kappers, C. U. 1921 Structural laws in the nervous system; the principles of neurobiotaxis. Brain, *44:* 125.

———— 1932 Principles of development of the nervous system. In Cytology and Cellular Pathology of the Nervous System. Edited by W. Penfield, Paul B. Hoeber, New York. *1:* 43–89.

Ariens Kappers, C. U., G. C. Huber and E. C. Crosby 1929 The Evolution of the Nervous System in Invertebrates, Vertebrates and Man. de E. F. Bohn, Haarlem. 335 pages.

BOEKE, J. 1932 Nerve endings, motor and sensory. In Cytology and Cellular Pathology of the Nervous System, edited by W. Penfield, Paul B. Hoeber, New York. *1:* 241–315.

BOK, S. T. 1915 Die Entwicklung der Hirnnerven und ihrer zentralen Bahnen. Die stimulogene Fibrillation. Folia Neurobiol., *9:* 475–565.

FISCHER, A. 1946 Biology of tissue cells. Cambridge University Press. 348 pages.

FULTON, J. F. 1943 Physiology of the nervous system. Oxford University Press, New York.

GRIGORIEFF, L. M. 1931–33 Differenzierung des Nervengewebes ausserhalb des Organismus. Arch. f. exp. Zellforsch., *11:* 483–519 and *13:* 195–220.

HARRISON, R. G. 1907 Observations on the living, developing nerve fibre. Proc. Soc. Exp. Biol. and Med., *4:* 140–143, and Anat. Rec., *5:* 116–118.

INGVAR, S. 1920 Reaction of cells to the galvanic current in tissue cultures. Proc. Soc. Exp. Biol. and Med., *17:* 198–199.

KARSSEN, A., AND B. SAGER 1934 Sur l'influence du courant électrique sur la croissance des neuroblastes in vitro. Arch. f. exp. Zellforsch., *16:* 255–259.

LEVI, G. 1919 Connessioni e struttura degli elementi nervosi sviluppati fuori dell'organismo. Mem. della R. Accad. dei Lincei, Series 5a, *12:* 142–182.

——— 1926 Richerche sperimentali sovra elementi nervosi sviluppati "in vitro." Arch. f. exp. Zellforsch., *2:* 244–272.

——— 1941 Nouvelles recherches sur le tissu nerveux cultivé in vitro; Morphologie, croissance et relations réciproques des neurones. Arch. de Biol., *52:* 133–278.

LEVI, G., AND H. MEYER 1945 Reactive, regressive, and regenerative processes on neurones, cultivated in vitro and injured with micromanipulator. J. Exp. Zool., *99:* 141–181.

LEWIS, W. H., AND M. R. LEWIS 1924 The behavior of cells in tissue cultures. In Textbook of General Cytology, edited by E. V. Cowdry, University of Chicago Press, Chicago, 383–447.

LUMSDEN, C. E., AND C. M. POMERAT 1951 Normal oligodendrocytes in tissue culture. Exper. Cell. Res., *2:* 103–114.

MARSH, G., AND H. W. BEAMS 1946 In vitro control of growing chick nerve fibers by applied electric currents. J. Cell. and Comp. Physiol., *27:* 139–157.

MOSSA, S. 1929 Ulteriori studi sulla rigenerazione dei neuriti e sulle modificazioni dei neuroblasti dei gangli spinali di embrioni di pollo coltivati "in vitro." Arch. f. exp. Zellforsch., *7:* 413–443.

MURRAY, M. R., AND A. P. STOUT 1947 Adult human sympathetic ganglion cells cultivated in vitro. Am. J. Anat., *80:* 225–273.

OLIVO, O. M. 1927a Migrazione di elementi nervosi coltivati in vitro. Arch. f. exp. Zellforsch., *4:* 43–63.

——— 1927b Differenziazione e sdifferenziazione del tessuto nervoso embrionale di pollo coltivati per piu settimane "in vitro." Arch. f. exp. Zellforsch., *5:* 46–76.

PARKER, G. H. 1929 The neurofibril hypothesis. Quart. Rev. Biol., *4:* 155–178.

PÉTERFI, T., AND S. C. WILLIAMS 1933 Elektrische Reizversuche an gezüchteten Gewebezellen. Versuche an Nervenzellen. Arch. f. exp. Zellforsch., *14:* 210–254.

———— 1934a Elektrische Reizversuche an gezüchteten Gewebezellen. Versuche an verschieden Gewebekulturen. Arch. f. exp. Zellforsch., *16:* 230–240.

———— 1934b Elektrische Reizversuche an gezüchteten Gewebezellen. Versuche an mischkulturen. Arch. f. exp. Zellforsch., *16:* 241–254.

POMERAT, C. M., J. R. EWALT, S. R. SNODGRASS AND M. F. ORR 1950 Tissue cultures of adult human cerebral cortex. Anat. Rec., *106:* 67–68, and Texas Rep. Biol. and Med., *8:* 108–110.

STRANGEWAYS, T. S. P. 1924 Tissue Culture in Relation to Growth and Differentiation. W. Heffer and Son, London. 60 pages.

WEISS, P. 1934 In vitro experiments on the factors determining the courses of the outgrowing nerve fiber. J. Exp. Zool., *68:* 393–448.

———— 1941 Nerve Patterns: Mechanics of nerve growth. Growth, *5:* 163–203.

———— 1944 In vitro transformation of spindle cells of neural origin into macrophages. Anat. Rec., *88:* 205–221.

———— 1945 Experiments on cell and axon orientation in vitro: the role of colloidal exudates in tissue organization. J. Exp. Zool., *100:* 353–386.

———— 1949 Differential growth. In The Chemistry and Physiology of Growth, edited by A. K. Parpart, Princeton University Press, Princeton, 135–186.

WEISS, P., AND A. C. TAYLOR 1944 Further experimental evidence against ''neurotropism'' in nerve regeneration. J. Exp. Zool., *95:* 233–257.

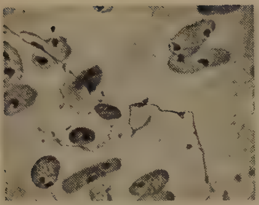
WILLIAMS, S. C. 1936 A study of the reactions of growing embryonic nerve fibers to the passage of direct electric current through the surrounding medium. Anat. Rec., *64:* no. 4, Suppl. 3, 56.

PLATE 1

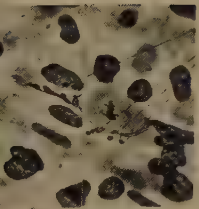
EXPLANATION OF FIGURE

2 Photographic reconstruction of complete neuroblast described in the text, ''p,'' perikaryon; ''a,'' the axone. The original photographs used for the measurements described in the text were printed at a magnification of × 2000. For purposes of reproduction, this figure has been reduced to × 429.

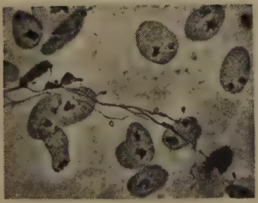
STUDY OF NEURITE
GANGLION



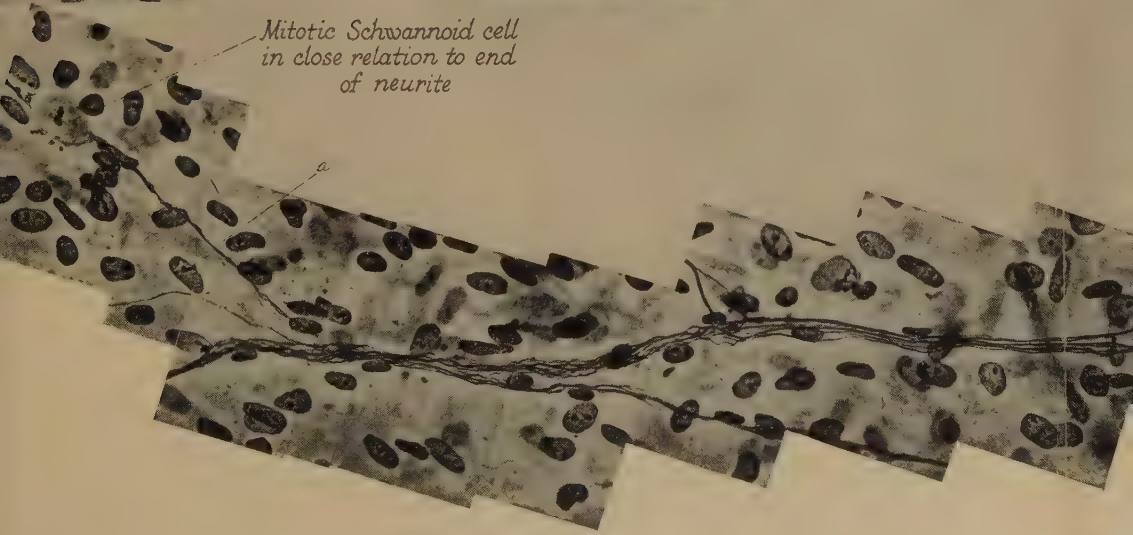
Quasi-synaptic relationship of terminals of two neurites in outgrowth



Terminals of neurite



"Boutons terminaux" on ends of neurites

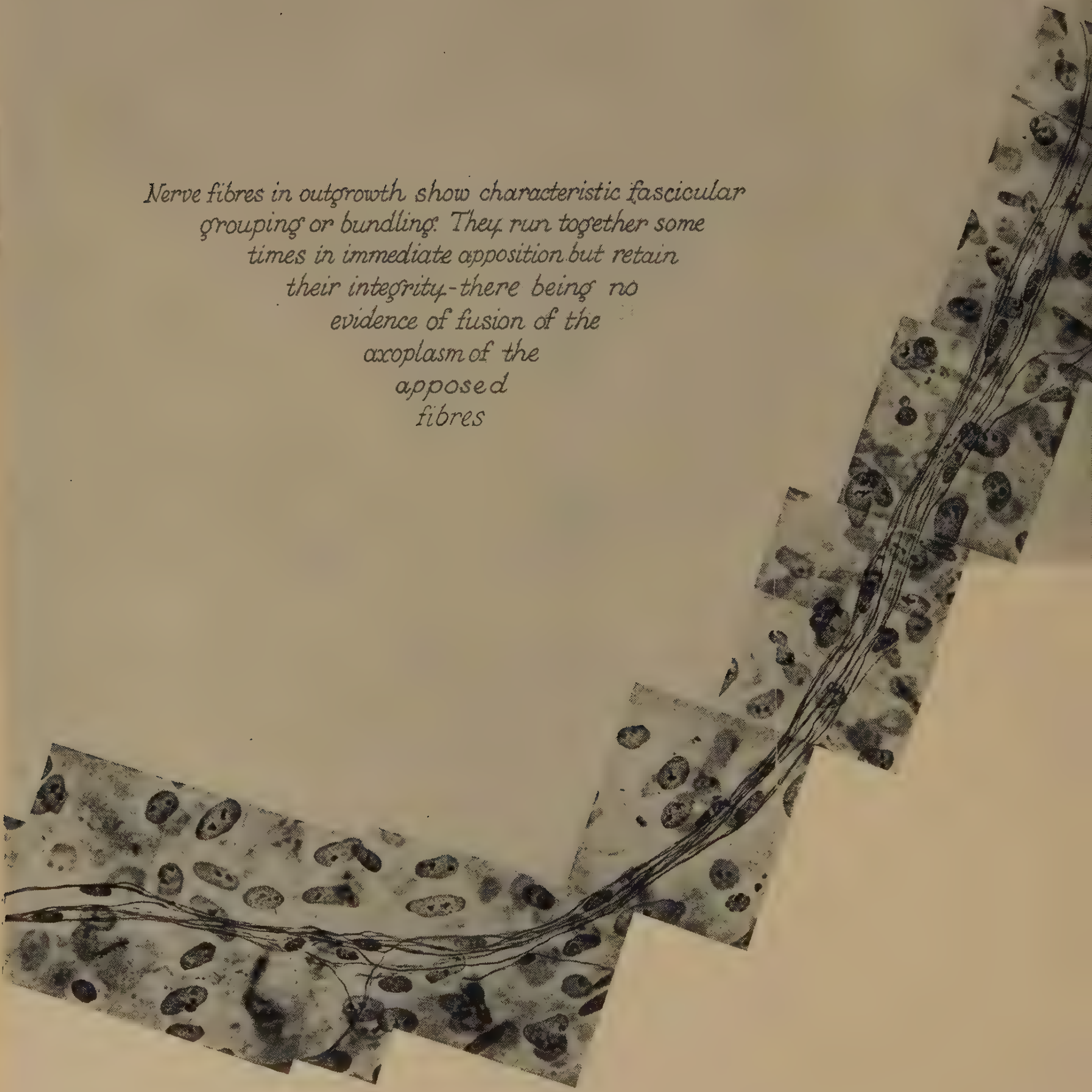


Mitotic Schwannoid cell in close relation to end of neurite

OUTGROWTH IN A WEEK-OLD OF A 9-DAY CHICK EMBRYO

MAGNIFICATION x2000

Nerve fibres in outgrowth show characteristic fascicular grouping or bundling. They run together sometimes in immediate apposition but retain their integrity-there being no evidence of fusion of the axoplasm of the apposed fibres



CULTURE OF POSTERIOR ROC

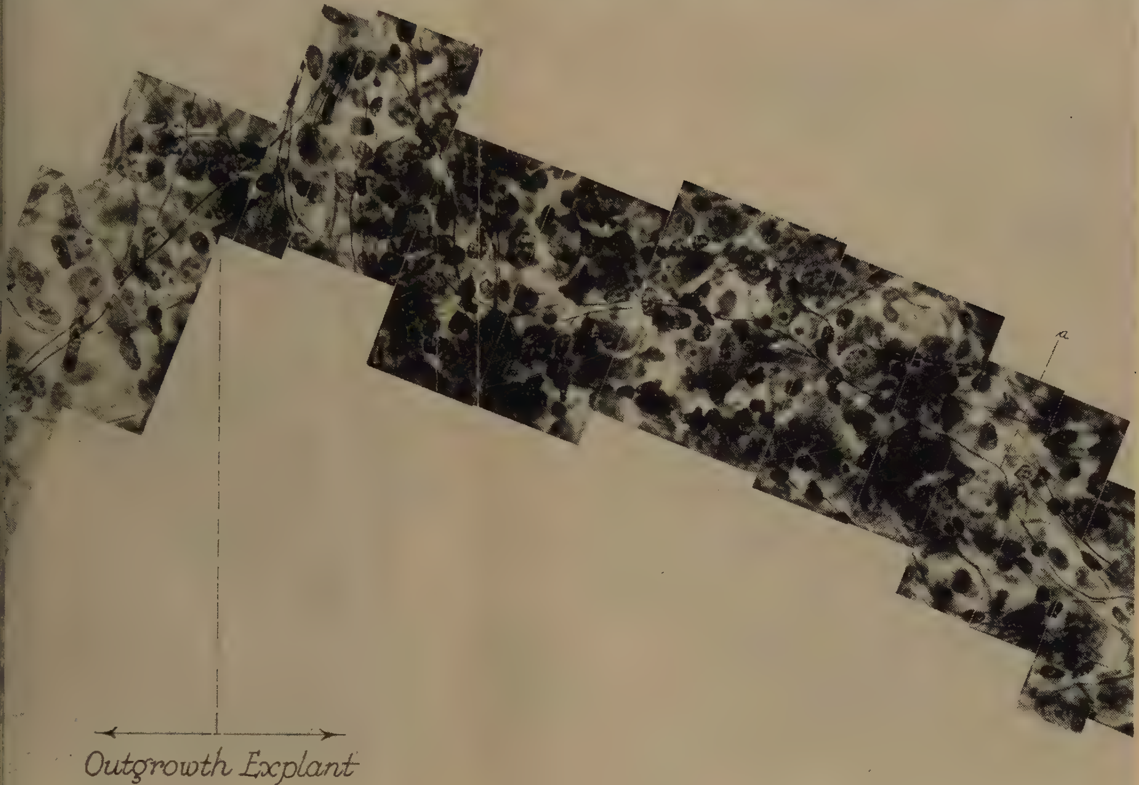
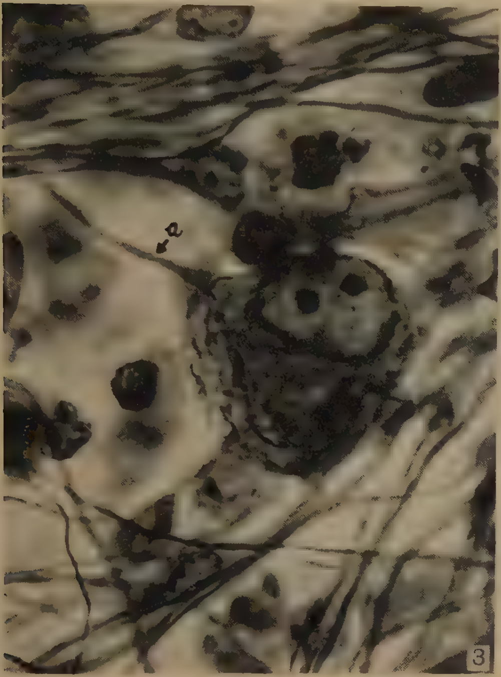
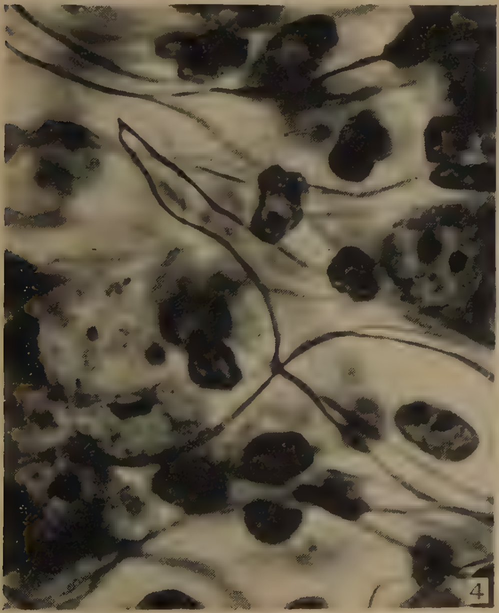


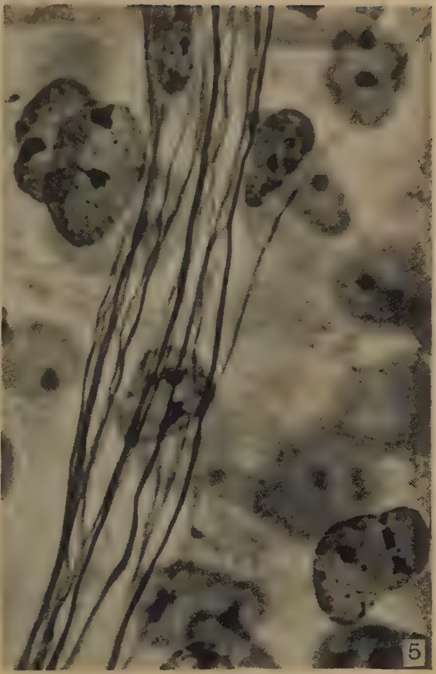
PLATE 1



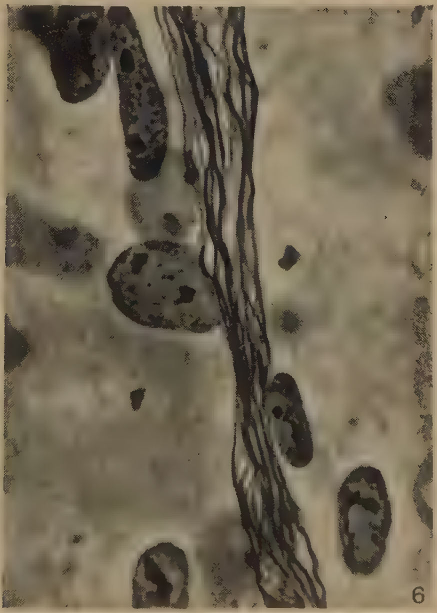
3



4



5



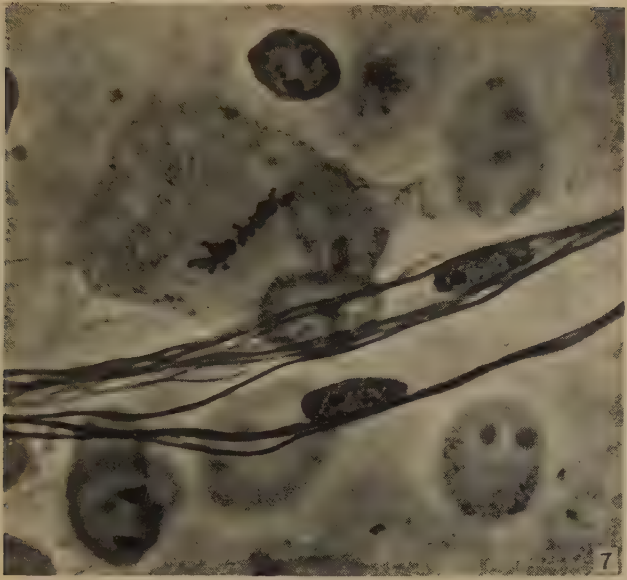
6

PLATE 3

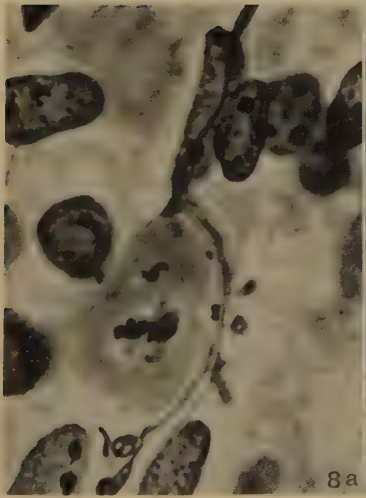
EXPLANATION OF FIGURES

7 Metaphase mitosis of a mesenchymal cell in vicinity of a neurite bundle. × 1429.

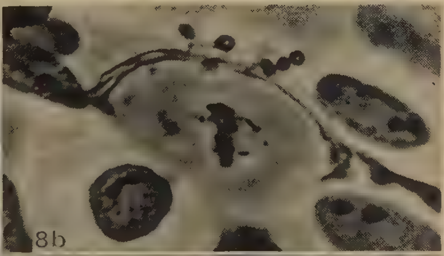
8 Schwannian cell in mitosis, in contact with terminal fibrils of the axone of the neuroblast illustrated in figure 2. For detailed description, see text. ''a,'' ''b,'' and ''c'' show detail at different focus. × 1429.



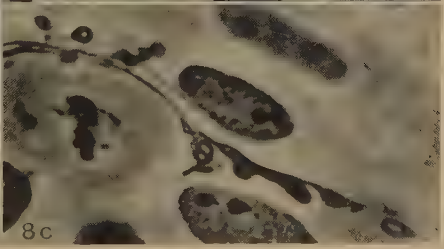
7



8a



8b



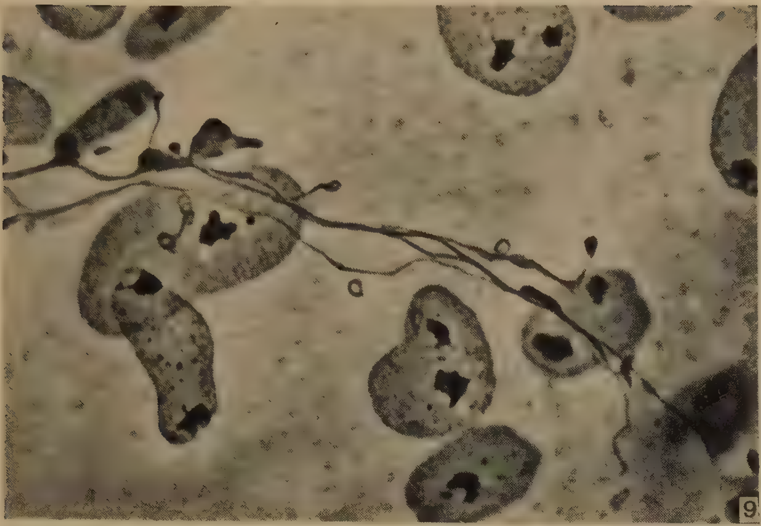
8c

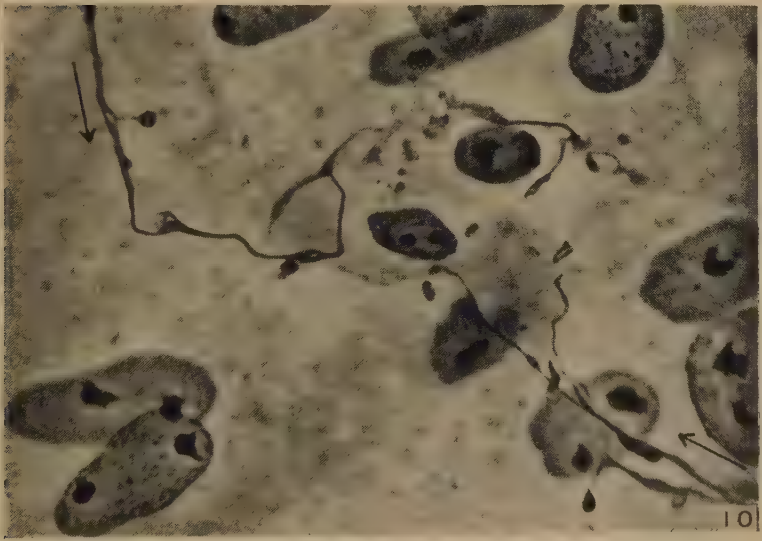
PLATE 4

EXPLANATION OF FIGURES

9 Rings and ''boutons'' on terminal filaments of axones in extreme periphery of zone of outgrowth. × 1429.

10 Quasi-synaptic relationship of terminal filaments of axones of two different neuroblasts. The arrows indicate the direction of growth. × 1429.


9


10

HISTOGENETIC INTERRELATIONSHIPS OF OOCYTES[1] AND FOLLICLE CELLS

A POSSIBLE EXPLANATION OF THE MODE OF ORIGIN OF CERTAIN POLYOVULAR FOLLICLES IN THE IMMATURE RAT

ALDEN B. DAWSON
The Biological Laboratories, Harvard University,
Cambridge, Massachusetts

THIRTEEN FIGURES

The possibility of the origin of polyovular follicles in the ovary of the rat by the subsequent oocytic differentiation of cells temporarily incorporated in the investing follicular epithelium was suggested by the chance observation of the young follicle illustrated in figure 3. In this preparation, stained by the periodic acid-leucofuchsin method of McManus, two cells located in the follicular epithelium had reacted selectively with the stain; one with an intensity equal to that of the cytoplasm of the enclosed oocyte. Such cells would accordingly furnish a possible source of accessory ova within a follicle provided their oocytic potentiality could be established. A survey of additional material was therefore undertaken to determine if supplementary evidence for this hypothesis could be obtained. Hartman ('26) has suggested three other possible modes of origin of polyovular follicles: by subsequent division of polynuclear ova; by fusion of previously separate follicles; by initial failure of separation of ova by ingrowing follicular cells. The last theory is the most commonly accepted one, especially in instances where the ova are of equal size. Where great disparity in size exists a secondary hypothesis of unequal rates of growth of ova has been invoked.

---

[1] To facilitate description the cumbersome but specific terminology, denoting the several stages in the developmental history of the egg cell, will not be used; the terms oocyte, ovum and egg cell are employed interchangably to designate any of these stages.

MATERIALS AND METHODS

The rats used in this study were from the colony maintained in our laboratory, a closely inbred strain, originally obtained from the Wistar Institute. The ovaries studied include several complete series taken at age intervals of one day, from birth to 25 days of age. Beyond this age the sampling, up to the 45th day, was at 5-day intervals. Serial sections of 8 ovaries from 80 day old rats and incomplete series of an unrecorded number of ovaries of mature rats of various ages, subjected to a variety of endocrine treatments, were also studied. Ovaries from 10 to 25 days of age were studied most intensivly as more significant departures from the typical organization of follicles were found to occur in this age period.

The observations on the early organization of atypical follicles are based chiefly on material fixed in Helly's fluid and stained by the periodic acid-leucofuchsin method of McManus, counterstained with either Weigert's or Delafield's hematoxylin, since this method gave most selective results. Differential extractions to separate glycogen and carbohydrate-protein complexes was not attempted. The relatively rare occurrence of these early atypical follicles made this seem impracticable. Furthermore, the histochemical studies of Harter ('48) on the rat ovary indicate that it would be extremely difficult to use the results as a means of discriminating between differentiating oocytes and possible potential oocytes incorporated in the follicular epithelium.

Bodian's protargol method (with gold toning) was also used since it selectively demonstrates early stages in oogenesis due to the presence of a diffuse flocculent argyrophilic reaction in the cytoplasm of the differentiating egg (Dawson, '49). However, in general this material appeared less reliable than Helly-fixed tissue due to occurrence of considerable shrinkage and distortion at the surface of the ovary where early stages of follicular organization most frequently occur.

Pap's silver impregnation method for reticulum, following fixation in Rossman's, Bodian's or Lavdowsky's fluids also proved extremely valuable in following differentiating follicles due to the sharp delineation of follicular capsules which are largely composed of densely interlaced reticular fibrils. Additional data on the incidence of obviously polyovular follicles were obtained from other series of ovaries fixed by a variety of routine methods which gave good preservation of the ova and granulosa cells.

DESCRIPTION

This study was begun as indicated in the introduction with the preconceived notion that typical follicular cells even as late as the unilaminar stage of follicular development (fig. 3) might under certain circumstances exhibit potentialities as oocytes — a potentiality theoretically lost when they failed to undergo rapid growth and became secondarily oriented about a growing egg cell. An examination of earlier stages (figs. 1 and 2), however, suggested that the condition observed in figure 3 might be the result of the incorporation into the follicular epithelium of cells which had already undergone an initial but incomplete differentiation into oocytes. Thus the ultimate fate of these cells might remain in the balance for some time. If they failed to differentiate further as oocytes and assumed the form and function of follicle cells it would then not be possible to follow their history further as they would no longer be distinguishable from neighboring follicle cells. On the other hand, if they differentiated further as egg cells they would probably exhibit retarded growth and should eventually appear within the follicle as accessory ova.

Follicles with accessory ova. Critical transitional stages are difficult to find. The best example observed is shown in figure 4, in which the smaller oocyte of this primary follicle lies in a peripheral position but is not completely aligned with typical follicle cells. In a slightly older follicle (fig. 5) the disparity in size of the chief and accessory ova is of the same order but in this folicle the situation is complicated by a constriction which partially isolates one accessory ovum from the main body of the follicle. The same relative size of chief and accessory ova may be maintained in still older follicles (figs. 6, 9 and 10). In such follicles the larger cell is centrally located while the smaller cell is eccentrically placed but is not in an extreme peripheral position. Ova of markedly unequal size have not been observed in follicles with antra.

Many of these atypical follicles exhibit evidences of early atresia but profound regressive changes are rare. Swelling of the follicle cells with disappearance of cell boundaries is relatively common. The nuclei are frequently enlarged and hypochromatic. Some pycnosis may be present but in many cases these degenerative changes are sharply contrasted by the presence of normal mitotic activity in adjoining cells.

In the follicle shown in figures 7 and 8, 4 small equal-sized ova are present. They are individually much smaller than the ovum should be in a uniovular follicle of this size and probably represent accessory ova. There is evidence of degeneration of a centrally placed ovum of unknown size.

Besides these accessory cells which are obviously egg cells, smaller densely-stained spherical cells are frequently encountered in older follicles. Their nuclei are similar to ordinary follicle cells but the cytoplasm is closely packed with fuchsinophile granules. Their maximum diameter is rarely greater than 12 μ. They most frequently lie in the antral space but may be in contact with the follicular epithelium. Many may be seen undergoing cytolysis in the follicular fluid. Smaller numbers of large, deeply stained granules are ordinarily present in the cells of cumulus and cells bordering the antrum (Harter, '48). Hartman ('26) interpreted these cells as degenerating granulosal cells and there is no evidence to contradict this conclusion, although superficially the resemblance between these granulosal calls and "primordial" ova is striking.

Follicles with ova of equal size. Biovular follicles with ova of equal size occur more frequently than those with ova of unequal size. The ova may be in direct contact with one another (fig. 11) or be separated by one to several layers of granulosal cells. These follicles appear to grow more successfully than those with ova of unequal size. The paired ova tend to occupy a central position in compact follicles, but their position becomes more peripheral with antrum formation and they may lie at considerable distances from one another.

Interfollicular bridging. The occurrence of follicles joined by solid epithelial cords (figs. 12 and 13) has already been recorded (Dawson and McCabe, '51). It was suggested that this condition was the result of herniation of follicular cells through the follicular capsule, brought about by the extra- and intrafollicular tensions developed in a rapidly growing ovary. It was further postulated that such extrafollicular cells constituted a probable source of the primary interstitial tissue characteristically present in the immature ovary from the 10th to the 18th day of life. The occurrence of the interfollicular bridges was interpreted as a result of random approximation and fusion of such secondary follicular extensions, which tended to persist once continuity of epithelial cells was secondarily established. These atypical follicles appear during the 6th and 7th days after birth and become most numerous about the 13th day but may occasionally be found as late as the 24th day. As many as 5 follicles have been found connected in a series, but many blind extensions also occur. While the present evidence appears to favor a secondary origin, the possibility that some of these atypical formations may represent varying degrees of persistence of the original ingrowing cords cannot be entirely excluded. The development of these linked follicles does not appear to be impaired as many possess antra. The general level of development of individual conjoined follicles appears comparable and the ova are equal in size.

Incidence of polyovuly in the rat

Reports of the occurrence of polyovular follicles in the rat are exceedingly rare although both immature and mature ovaries have been extensively studied by numerous investigators. Hartman ('26), in a comprehensive review of the literature, noted with surprise the absence of rat from the list of animals exhibiting this phenomenon. Since that time a few investigators have reported polyovular follicles. Lane ('38) has given the most complete account for the laboratory rat. During the examination of 100 ovaries from 60 rats which varied in age from 15 to 64 days, 21 atypical follicles (5 with binuclear ova, 13 biovular, and three triovular) were found. All the binuclear follicles were observed in ovaries of 4 rats aged 22, 28 and 33 days. The polyovular follicles were more evenly distributed, usually occurring once per ovary. Apparently all the ova of an individual follicle were equal or nearly equal in size as Lane did not refer to any disparities in this respect.

Marx ('41) has reported one case of polyovuly in an adult animal. More recently Davis and Hall ('50) have given data on the occurrence of polyovuly in mature, wild Norway rats. Forty-seven out of 394 animals, or 12% of the rats (6% of the ovaries) examined had at least one polyovular follicle. All of these were apparently biovular and presumably the ova within each follicle were of equal size since no comment to the contrary was made.

Our observations, both on age distribution and frequency of occurrence of atypical follicles, are in general agreement with the findings of Lane ('38). Biovular follicles with ova of equal size occurred most commonly but tended to be limited to follicles without antra. Follicles with ova of unequal size were less numerous and early stages in which a presumptive oocyte was identified in the follicular epithelium were quite rare. No attempt has been made to treat this problem statistically as our chief interest centered about an analysis of the processes which resulted in the development of atypical follicles. The number of aberrant follicles recognized appeared, in many instancs, to be a function of the number of times a series of sections was reexamined.

Certainly atypical follicles were most numerous during the age period from 10 to 25 days. None were observed before the 10th day and they were rare after the 25th day. Only biovular follicles with ova of equal size were found in sexually mature animals — a total of 8 being noted in the ovaries examined. Of these, 5 were compact follicles and the other three possessed antra. In the limited age period in which they are commonly found, the follicles with epithelial bridges were much more numerous than all the types of atypical follicles recognized. This suggests that this phenomenon is not directly related to the formation of polyovular follicles.

DISCUSSION

Polyovular follicles have usually been regarded as due to failure of the morphogenetic process after the potentialities of the derivatives of the germinal epithelium, in terms of ova and follicle cells, have been finally restricted; i.e., they represent merely imperfections in the arrangement of cells whose fates have already been determined. In many cases, especially where the contained ova are of normal and equal size, this is probably a correct interpretaton. Great disparities in egg cell size were then attributed to arrest of growth of certain ova or to their unequal rates of growth. The alternative possibility that disparity in egg cell size might stem from tardy or incomplete restriction of the potentiality of the derivatives of the germinal epithelium has not been seriously considered. Just how much such a delay, if it does occur, would handicap a cell in the future realization of its development as an ovum is of course problematical.

The evidence here presented is at best suggestive; it is certainly not conclusive. Both selective staining as a means of identification of individual cell types, and the arrangement of a variety of stages into a morphological series to represent a picture of progressive development have their obvious weaknesses. The stages of early follicular development illustrated admit two interpretations. In these follicles, elements whose cytoplasmic differentiation is apparently oocytic occupy the position of follicle cells in relation to a definitive ovum. Some may be partially differentiated oocytes temporarily diverted to a follicular cell positon to eventually appear as accessory ova. Others may have been undistinguishable from potential follicle cells when originally incorporated into the follicular epithelium and later exhibited oocytic potentiality while retaining for a time both the position and form of a follicle cell. In the absence of any continuous history the ultimate fate of such cells must also remain in doubt.

The chief and accessory ova of polyovular follicles exhibit size differences to varying degrees. Two extreme examples are illustrated by Hartman ('26, figs. 7a and 7b). In these the large chief eggs are centrally located with very small accessory ova occupying an extreme peripheral position in the granulosa. Hartman regards these as evidences of the different properties of individual cells which either grow or remain retarded as the case may be. The present paper attempts to relate these differences to the early histogenetic relationships of egg and follicle cells and the progressive restriction of potentialities which occurs in association with follicular organization.

This problem has recently received considerable elucidation from the rather remarkable observations of Gaillard ('50) on the behavior of explants of the foetal human ovarian cortex. Gaillard found that, besides the cord-like structures which originated from the surviving germinal epithelium, a second system of anastomosing cords developed from the follicle cells of primordial follicles in which the oocytes had degenerated after explantation. The organization of persisting follicle cells into branched cords occurred only in explants of older fetuses (20–26 weeks old). Within these follicular cords new oocytes developed and, although there were numbers of cells of follicular origin surrounding these ova, never more than one layer was organized about an ovum as a definitive follicular epithelium. It also appeared that the formation of these primordial follicles was more or less independent of the degree of nuclear differentiation of the egg cells. The degree of cytoplasmic differentiation was also variable. In some ova the cytoplasm was poorly developed, in others a "fine vitellogenesis" had occurred.

These observations emphasize the significance of the intimate histogenetic relationships between the germinal epithelium, the egg cells and the inital layer of follicle cells. The failure to form a stratified granulosa in any of the follicles suggests that conditions of the experiments were not favorable for extensive growth of the newly organized follicles. Basically, Gaillard's observations support the concept of oocytic potentiality of follicle cells postulated in this study, at least during the period in which they exist in the form of a single layer of cells. In fact this is the fundamental vertebrate plan since a simple follicular epithelium is ordinarily uniformly present below mammals. Even in mammals the newly organized unilaminar follicle is quickly delimited from the surrounding tissue by a follicular capsule and the multilaminar condition is secondarily developed by rapid proliferation and reorientation of the cells within this segregated unit, not by addition of cells to the periphery. This suggests that the interacting system — ovum and follicle cells — has initially a very limited range (Hisaw, '47; Vincent and Dornfeld, '48). The additional interactions that result in the formation of a stratified granulosa and the eventual appearance of an antrum, together with the organization of extrafollicular thecae, cannot be readily recognized. At some point in the evolution of the follicle these interacting components become subject to external influences that are primarily hormonal in origin.

It is not the intent of this paper to advance the hypothesis outlined to the exclusion of other modes of origin of polyovular follicles. It seems most applicable to follicles in which chief and accessory ova are present. Other theories will not be discussed here. Most of the likely possibilities have been critically canvassed, earlier by Hartman ('26) and Mainland ('27), later by Harrison ('48) and Desaive ('49). One general conclusion may be drawn. Polyovular follicles with ova of equal size are most likely to survive, and of these, biovular follicles appear to have a distinct advantage.

SUMMARY

Special attention has been directed to polyovular follicles in which there is great discrepancy in the size of the contained ova, a disparity so great that the terms chief and accessory ova are frequently employed. Evidence is presented in favor of the view that these atypical follicles either result from the temporary incorporation within the follicular epithelium of retarded cells whose potentialities as future egg cells is fully determined or are produced by the subsequent oocytic differentiation of apparent follicle cells whose potentialities were not finally restricted at the time they became specifically oriented about a definitive ovum. A study of transitional stages suggests that the oocytic potentialities of such presumptive follicle cells is limited to the period in which they exist as a simple layer about the ovum. The experimental studies of Gaillard ('50) on the human ovarian cortex further emphasize the intimate and significant histogenetic interrelationships of derivatives of the germinal epithelium, egg cells, and the first layer of follicle cells.

Polyovular follicles with ova of equal size are regarded as due to failure of the morphogentic process after the potentialities of the derivatives of the germinal epithelium, in terms of ova and follicle cells, have been finally restricted.

Other atypical follicles joined by epithelial bridges (follicle cells) are interpreted as secondary formations, neither residues of ovigerous cords nor stages in the fusion of follicles.

LITERATURE CITED

Davis, D. E., and Octavia Hall 1950 Polyovuly and anovular follicles in the wild Norway rat. Anat. Rec., *107:* 187–192.

Dawson, A. B. 1949 Argyrophilic inclusions in the cytoplasm of the ova of the rat. Anat. Rec., *103:* 568–569 (abstract).

Dawson, A. B., and Marcia McCabe 1951 The interstitial tissue of the ovary in infantile and juvenile rats. J. Morph., *88:* 543–572.

Desaive, P. 1949 Étude des follicules a ovocytes doubles ou multiples dans l'ovaire de lapine adulte. Arch. Biol., *60:* 357–407.

Gaillard, P. J. 1950 Sex cell formation in explants of the foetal human ovarian cortex. Proc. Kon. Ned. Akad. v. Wetensch. *53:* 1300–1316; 1337–1347.

HARRISON, R. J. 1948 The changes occurring in the ovary of the goat during the estrous cycle and in early pregnancy. J. Anat., *82:* 21–48.

HARTER, B. T. 1948 Glycogen and carbohydrate-protein complexes in the ovary of the white rat during the estrous cycle. Anat. Rec., *102:* 349–367.

HARTMAN, G. G. 1926 Polynuclear ova and polyovular follicles in the opossum and other mammals, with special reference to the problem of fecundity. Am. J. Anat., *37:* 1–51.

HISAW, F. L. 1947 Development of the Graafian follicle and ovulation. Physiol. Rev., *27:* 95–119.

LANE, C. E. 1938 Aberrant ovarian follicles in the immature rat. Anat. Rec., *71:* 243–247.

MAINLAND, D. 1927 The pluriovular follicle, with reference to its occurrence in the ferret. J. Anat., *62:* 139–158.

MARX, LORE 1941 Replacement of ovocytes in the ovary of normal and hormone-injected young rats. Anat. Rec., *79:* 115–131.

VINCENT, W. S., AND E. J. DORNFELD 1948 Localization and role of nucleic acids in the developing rat ovary. Am. J. Anat., *83:* 437–469.

PLATES

DESCRIPTION OF PLATES

All figures are photomicrographs of ovaries sectioned at 5 μ. The tissues of the first 11 figures were fixed in Helly's fluid, stained by the periodic acid leucofuchsin method of McManus and counterstained with either Delafield's or Weigert's hematoxylin. The cytoplasm of the ovum, the zona pellucida, and certain cells (potential oocytes or follicular cells?) associated with the organization of the primary follicle are stained an intense red. In figures 11 and 12 the tissues were fixed in Bodian's aceto-alcohol-formal mixture, impregnated by Pap's method for reticulum and counterstained with Mallory's triple stain. The follicular membranes were sharply blackened.

PLATE 1

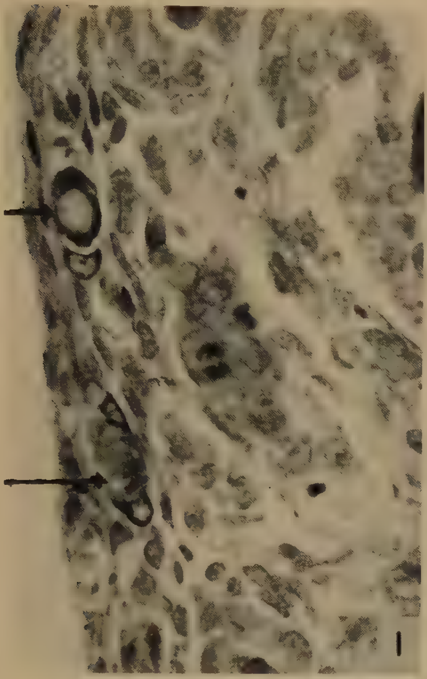
EXPLANATION OF FIGURES

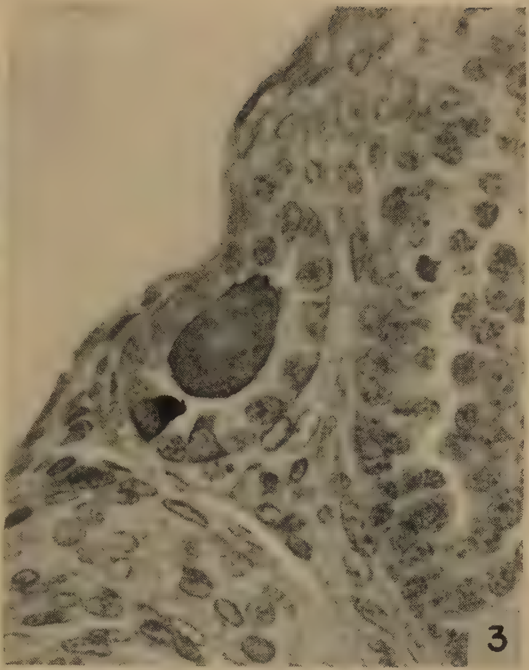
1 Two early stages in the organization of primary follicles. In the lower follicle two densely stained cells are shown in close association with a tangentially sectioned oocyte but they are not significantly larger than neighboring cortical stromal cells. In the upper follicle a somewhat larger, heavily stained cell is located at the lower pole of the oocyte with a normal, flattened follicular cell at the opposite pole. Rat, 13 days old, × 825.

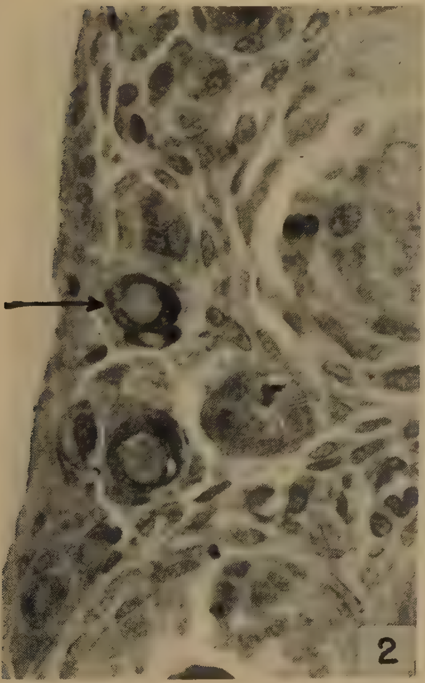
2 A primary follicle showing an oocyte completely invested by a layer of flattened cells. The continuity of typical follicular cells is interrupted by the presence of a large, densely stained cell. Rat, 13 days old, × 825.

3 A later primary follicle with cuboidal follicular cells. Two cells whose shape and position are characteristic of follicular cells have reacted strongly with leucofuchsin. Rat, 25 days old, × 825.

4 A biovular follicle containing two oocytes of unequal size. The follicle is essentially unilaminar although the presence of the second oocyte has produced some irregularity in the arrangement of the follicular cells. Rat, 10 days old, × 825.


1


3


2

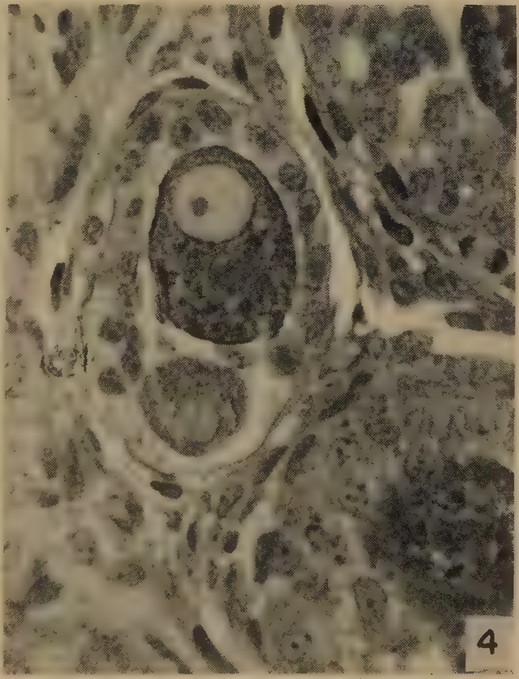

4

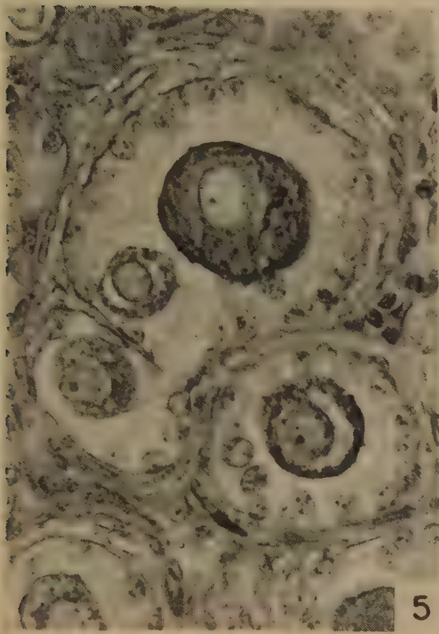
PLATE 2

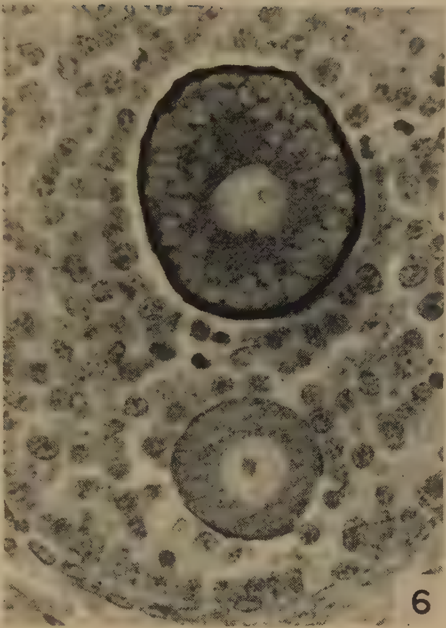
EXPLANATION OF FIGURES

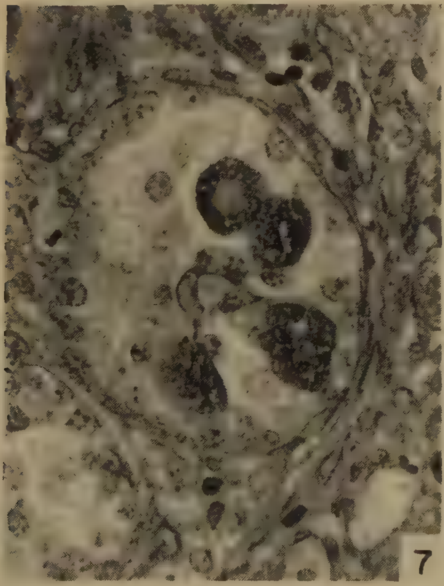
5 An irregularly shaped, triovular follicle with one large and two small, equal-sized oocytes. The cytoplasmic limits of the follicular cells are poorly defined and their nuclei are hypochromatic. Rat, 17 days old, × 620.

6 A multilaminar secondary follicle (without antrum) contained two oocytes of unequal size. The follicular cells appear normal and many mitotic figures were observed. Rat, 25 days old, × 620.

7 and 8 Successive sections of a small secondary follicle showing the presence of 4 equal-sized oocytes. The center of the follicle was apparently occupied by a 5th oocyte of which only vestiges remain. The follicular cells are indefinitely outlined and many of their nuclei are enlarged and hypochromatic. Rat, 17 days old, × 620.


5


6


7

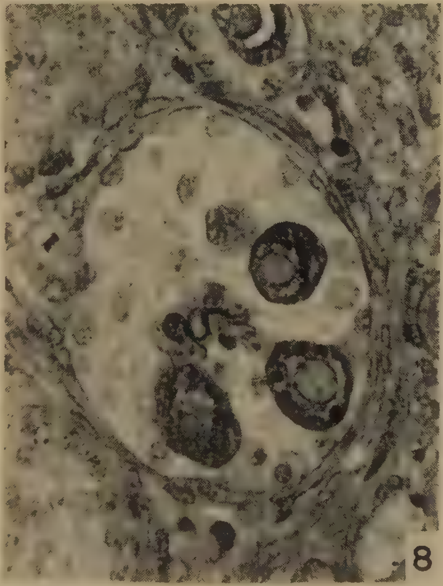

8

PLATE 3

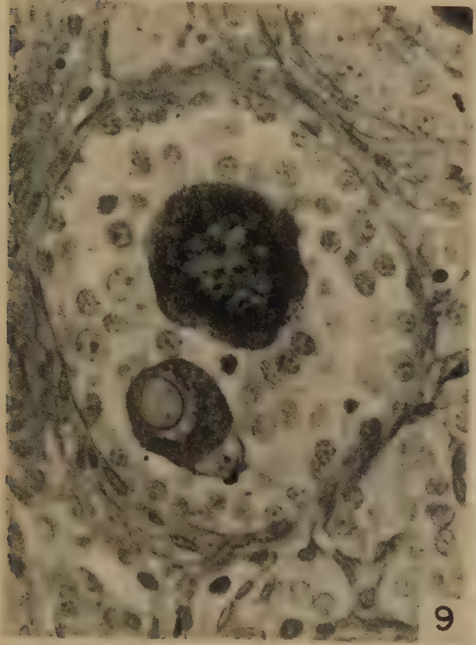
EXPLANATION OF FIGURES

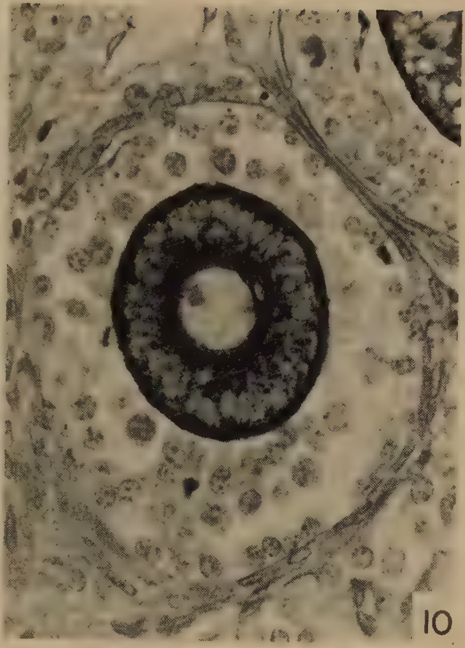
9 and 10 Two sections (20 μ apart) through a secondary biovular follicle showing the difference in size of the two oocytes. The granulosa appears to be undergoing regressive changes. The boundaries of many follicular cells are poorly outlined, with enlarged and hypochromatic nuclei. Seven pycnotic nuclei and 4 normal mitotic figures were also observed in this follicle. Rat, 23 days old, × 620.

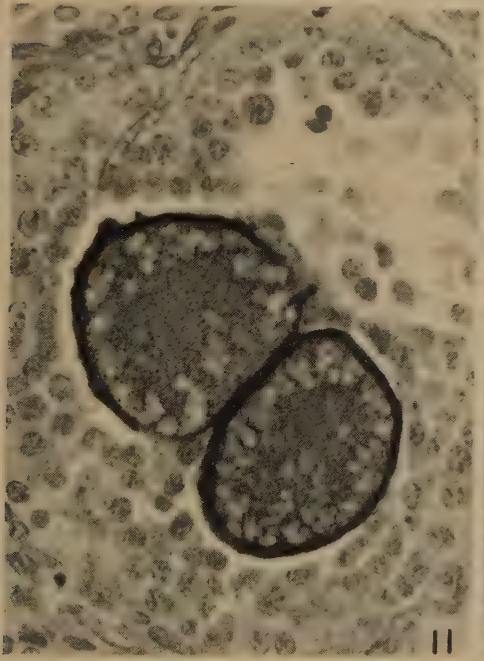
11 A vesicular follicle with an early antrum, containing two contiguous oocytes of equal size. The follicular capsule is indented at one point. Rat, 25 days old, × 620.

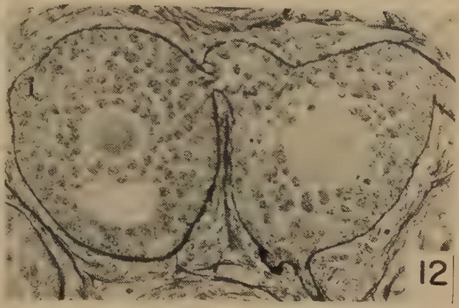
12 Two follicles joined by a narrow granulosal bridge. The three additional processes shown extended varying distances but all ended blindly. Rat, 12 days old, × 220.

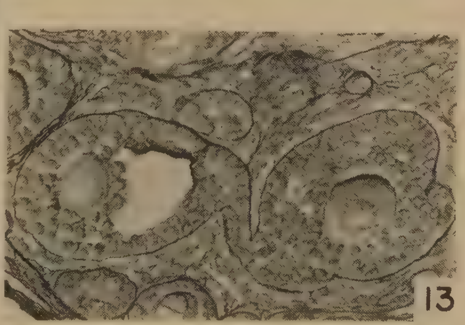
13 Two similar, conjoined follicles. These follicles also possessed 5 additional bud- or cord-like processes, not evident in the illustration; two joined other neighboring follicles and the remaining three ended blindly. Rat, 13 days old, × 220.


9


10


11


12


13

THE SKULL AND THE DENTITION OF SCREW TAIL MICE

SURINDAR NATH BHASKAR, ISAAC SCHOUR, *EDWIN CARLETON MacDOWELL AND JOSEPH PETER WEINMANN

*Departments of Histology and Oral Pathology, College of Dentistry, University of Illinois, Chicago; and *Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island, New York*

THIRTY-ONE FIGURES

INTRODUCTION AND REVIEW OF THE LITERATURE

Lannes and MacDowell in 1941 reported the appearance of a recessive mutation in the 50th generation of brother and sister inbreeding of CSH Bagg Albino mice. Because of the coiled tail at birth, the name "screw tail" (symbol "*sc*") was given to this mutation. However, the coiling of the tail was not the only abnormality. Deviations from the normal have been noticed in the pelvis, sternum, cranial sutures, vertebrae, and the dentition.

MacDowell, Potter et al. ('42) described in detail the abnormalities observed by gross anatomical study. Both the endochrondral and membranous bones were affected. They ascribed the coiling of the tail at birth to shortness of the tendons, and the kinks in the vertebral column to shortened, defective centers of individual vertebrae. The pelvis was found to be placed approximately one vertebra nearer the tail. The sternum, instead of being long and composed of 6 small segments, was wider, shorter and unsegmented. The long bones were shorter than normal.

The cranium showed anomalies of the coronal and the frontal sutures. The mandible was overdeveloped in its anterior part and shortened in the antero-posterior direction. There was malocclusion of the teeth, deficient growth of the molar roots, and absence of backward growth of the basal end of the lower incisor. A mortality rate of 60% in the first three days after birth was closely connected with inability to suckle. Even survivors did not take a normal amount of milk.

A detailed embryological analysis of the *sc* sternum led Bryson ('45) to suggest that most of the developmental disturbances in the *sc* mice could be explained by deficient growth of the mesodermal tissues.

The purpose of the present paper is to describe in greater detail the cranium, the maxilla, the mandible, and the dentition of *sc* mice, and of their normal litter mates, and to compare the anomalies produced by the *sc* gene with those previously produced by nutritional and hormonal deficiencies.

MATERIAL AND METHODS

Screw tail mice, being sterile, are obtained together with normals, from normal parents carrying one dose of the *sc* gene. The normal litter mates provide not only a control on age but also on the specific action of a double dose of this gene in *sc* mice, since with exclusively inbred ancestry this gene is the only genetic variable.

The present study is based on the roentgenologic and histologic investigation of 58 skulls of highly inbred CSH Bagg Albino mice, varying in age from 14½ days in utero to 420 days. Of these, 34 were of *sc* mice and 24 of normal mice born in the same litters. Table 1 gives the number and ages of the control and *sc* animals. Dorso-ventral and lateral roentgenograms were taken of 29 skulls, varying from 0 to 80 days of age. The histologic investigations are based on complete serial sections, in longitudinal or frontal direction, of 44 skulls. The skulls, after routine decalcification and embedding in celloidin, were sectioned and stained with hematoxylin and eosin.

In addition, gross anatomic studies were made on 8 *sc* skulls and a number of normal skulls varying in age from 26 to 420 days.

OBSERVATIONS

Cranium

The skulls of the *sc* mice were smaller than those of the litter mate controls (figs. 1, 2). As measured on roentgenograms, the greatest inner height of the cranium, that is, the distance between the base of the cranium and the highest point of the vault, was about 6 mm in 15 day old control mice. It remained constant from the 15th day to the 80th day. The cranial vault was convex up to the 15th day of life. After the 15th day, it flattened out and remained flat from then on. In the *sc* mice, the inner height of the skull was the

TABLE 1

Age and number of control and sc skulls studied roentgenologically and histologically

AGE	DORSOVENTRAL AND LATERAL ROENTGENOGRAMS		SERIAL, FRONTAL AND LONGITUDINAL HISTOLOGIC SECTIONS	
	No. of control animals	No. of *sc* animals	Control	*sc* animals
$14\frac{1}{2}$ d.i.u.			1	1
15 d.i.u.			2	2
16 d.i.u.			1	1
17 d.i.u.			1	1
Birth	1	1	2	2
1 day	1	1	1	2
2 days	1	1	2	2
3 days	1	1	2	2
4 days	1	1	2	2
6 days	1	1	2	2
10 days	1	1	1	1
12 days	1	1	1	1
15 days	1	1	1	1
20 days	1	1	1	1
30 days	1	1	1	1
40 days	1	1		1
50 days	1	2		
80 days	1	1		
Totals	14	15	21	23

same as in the controls, but its length and width were reduced and the vault of the cranium remained convex even at 80 days of age.

In roentgenograms, the occipital and the tympanic bulla bones could be identified in the controls at the 4th and 6th day, respectively, and in the *sc* mice between the 6th and 10th days. The cranial vault (frontal, parietal, interparietal and occipital bones) of an 80 day old control mouse consisted of an outer and an inner plate of bone. The suture between the bones showed almost end to end relationship. In the *sc* mouse of the same age, the bones of the cranial vault appeared as single bony plates and, at the fronto-parietal suture, the parietal bone overlapped the frontal. The histologic study of these bones confirmed and extended the roentgenological findings given above. The sections showed that in the controls the frontal and parietal bones near the coronal suture consisted of single plates of bone up to the 15th day of life. The parietal bone overlapped the frontal and was separated from it by actively proliferating connective tissue. In 20 day old animals, however, both of these bones showed signs of the beginning formation of a second plate of bone near the coronal suture, and in 30 day old control animals both the frontal and parietal bones near the coronal suture consisted of two plates. Between the bony plates, red bone marrow could be seen in 20 day old specimens (fig. 26). The outer and inner plates of the parietal and frontal bones now met end to end.

In young specimens of *sc* mice, the relation between the frontal and parietal bones was the same as in the controls, that is, the parietal and frontal were single plates, and the former overlapped the latter. This relation, however, remained unchanged in the *sc* mice, as shown by the oldest histologic specimen sectioned (50 days), and as observed by the x-ray (80 days; fig. 2). Thus, neither the second plates of bone nor red marrow developed (fig. 27).

Mandible

The control and *sc* mandibles began to show differences in size and shape between the 10th and 12th days. From this time on, the *sc* mandible appeared increasingly malformed. Length, and height, of the *sc* mandible were smaller than in the controls, as were also the ramus, condyle, coronoid and angular processes. The condyle was distorted, rough, and irregular in outline. The mandibular foramen was closer to the lower border of the mandible than in the controls, and this difference became more marked with age.

Histologic sections showed that the *sc* mandible was not only shorter than the control mandible, but that it was also short for the *sc* maxilla and premaxilla. This was apparent from the relation of the tongue to the upper incisor. Whereas in the normal relation of maxilla and mandible, the tongue is directly under, or in contact with, the alveolar ridge of the upper incisor, in the *sc* skull there was a wide space between the two. (Compare figs. 5 and 6.)

In contrast to the poorly developed ramus of the *sc* mandible, the body was relatively increased in size below and anterior to the lower first molar. The disproportionate size of the anterior part caused a sharp notch in the lower border of the mandible. This notch was a constant feature of the lower jaw of the *sc* mice.

Condyle

In the *control* animals, the condyle at birth consisted of cartilage. On its articular surface, it was covered by a layer of fibrous connective tissue. Below this layer, the following zones of cartilage cells could be seen:

1. A transitional zone of cells in which the fibroblasts had differentiated into flat, oval chondrocytes and were arranged with their long axis parallel to the articular surface.
2. A zone of maturing chondrocytes. Here the chondrocytes were spherical and showed vacuoles in the cytoplasm. There was more intercellular substance than in zone 1.

3. A zone of calcified cartilage matrix and of edematous chondrocytes. Here there was less intercellular substance, and it stained a deep blue with hematoxylin. The chondrocytes were enlarged, and in the deepest layers their capsules showed erosion by cells of invading young connective tissue.

Below this area of cartilage destruction, the so-called cartilage front, remnants of calcified cartilage could be seen. Around these, bone was being deposited. Trabeculae close to the cartilage front, the so-called primary spongiosa, and trabeculae at some distance from the cartilage front, the so-called secondary spongiosa, could be seen at birth. Below the secondary spongiosa, hemopoietic bone marrow could be seen.

In control animals, the thickest part of the cartilaginous condyle consisted at birth of 34 to 52 layers of cells; at 2 days of 30 to 40 layers; at 3 days of 30 to 37; and at 6 days of from 23 to 31 layers of cells. From 6 to 30 days of age, the thickness of the condylar cartilage decreased more gradually, but its width increased progressively. The condyle at 30 days of age was mushroom shaped with a covering of flattened condylar cartilage (fig. 30). Remnants of calcified cartilage matrix could be seen both in the primary and the secondary spongiosa, forming the core of the trabeculae. Primary and secondary bone trabeculae were abundant in the neck of the condyle. The red bone marrow increased in amount with age, but was always seen at some distance from the cartilage front.

In the *sc* condyle, all the zones present in the normal condylar cartilage could be seen, but the number of cell layers in each zone was reduced (compare figs. 28, 29). Thus at birth, the cartilage at its thickest part consisted of 22 to 35 layers of cells, at 2 days of 18 to 25, at 3 days of 16 to 22, and at 6 days of age of 9 to 17 layers of cells. Between 6 and 30 days of age, the *sc* condylar cartilage, like that of the control, continued to decrease in thickness and increase in width. However, it was always thinner and narrower than in the comparable control. At 30 days of age (fig. 31) it was hemispherical instead of flattened as in the control.

Primary and secondary bone trabeculae in the *sc* condyle were thicker and fewer in number, and the hemopoietic bone marrow closer to the cartilage front than in the control condyle of the same age. These differences were already apparent at two days of age, and increased as the animal grew older. In the 6-day-old *sc* mouse, the secondary spongiosa was reduced to one or two thick trabeculae of bone or was completely missing. Later, the area normally occupied by secondary spongiosa was filled with red bone marrow, and the elements of hemopoietic bone marrow could be seen even between the trabeculae of the primary spongiosa or next to the cartilage front.

Premaxillo-maxillary suture

Histologic sections showed that in the *control* animals at one day of age, the premaxillo-maxillary suture consisted of 4 to 7 interdigitating projections of premaxilla and maxilla. The maximal depth of this interdigitation was 0.25 mm (fig. 22).

The two bones were joined by a strip of connective tissue in which three zones could be distinguished, a central zone in which the cells were small and closely packed and two peripheral zones, in which the cells were larger and separated by more abundant connective tissue fibers. At the tip of the projecting trabeculae, osteoblasts and active bone apposition could be seen.

With increasing age, the interdigitation of premaxilla and maxilla became progressively deeper (fig. 24) so that at 20 days of age the maximal depth of the projections was about 1.4 mm. The connective tissue of the suture increased in amount, and its three layers mentioned above became more clearly demarcated. The connective tissue fibers in the two peripheral zones increased in abundance.

In *sc* mice at one day of age, the premaxillo-maxillary suture consisted of 3 to 5 alternating processes which did not interdigitate (fig. 23). The connective tissue of the suture did not differentiate into the three layers seen in the controls. The two peripheral zones seemed to be absent; the sutural tissue throughout contained closely packed connective tissue cells with very few intercellular fibers. Only at 12 days and later was there a tendency towards differentiation into the normal three zones. However, this differentiation was never as marked as in the normal.

Although osteoblasts and osteoid tissue could be seen at the tip of the alternating projections in one-day-old *sc* mice, there was complete lack or only a very slight degree of sutural interdigitation between 10 and 30 days of age (fig. 25). At the age of 20 days, the maximal length of the alternating processes was about 0.15 mm, as compared to 1.4 mm in the controls. The trabeculae and the inter-trabecular spaces were narrower than normal, and the bones at the sutures were thinner.

Maxillo-pterygoid suture

As in the premaxillo-maxillary suture, interdigitation and differentiation of the sutural connective tissue into three zones was lacking in the maxillo-pterygoid suture of the mutant mouse.

Incisors

Roentgenograms showed that in comparison to the controls, the upper and lower incisors of the *sc* mice were placed farther forward in the jaws and that they formed smaller segments of narrower spirals (figs. 1, 2). They showed malocclusion, or lack of occlusion, and the resulting continuous lengthening of their extra-alveolar parts. The basal regions of both the upper and lower incisors showed accordion-like folding.

Upper incisor — control. In mice, the alveolus of the upper incisor at the time of functioning is formed by parts of the premaxilla and parts of the maxilla. These bones are joined by the premaxillo-maxillary suture (fig. 3). The incisor starts its development in the premaxilla and gradually grows posteriorly into the maxilla.

At birth and at one day of age, histologic sections showed that the upper incisors were still situated in the premaxillary bone (fig. 7). The premaxilla, however, did not completely enclose the basal end of the incisor, but was perforated at the center of the fundus. Here the connective tissue of the suture was in contact with the connective tissue of the pulp of the incisor. Large numbers of multinucleated giant cells (osteoclasts) could be seen on the surface of the fundic bone facing the incisor. In mice two and three days old, resorption of bone had progressively enlarged the opening in the premaxillary part of the fundus, and the basal end of the incisor grew backward.

At the age of 4 days, the basal end was situated in the maxilla, and most of the fundus was formed by that bone (fig. 9). From this time on, the maxillary bone took an increasingly greater share in the formation of the socket.

With the backward growth of the incisor, the plane of the epithelial diaphragm and fundic bone showed changes in inclination. At birth, these parts were inclined backward and considerably upward. The upward inclination decreased gradually, and at about the 10th to 12th day the diaphragm and the fundic bone occupied an almost horizontal position (fig. 5). Between the 12th and 30th day, the basal end of the incisor, the epithelial diaphragm, and the fundic bone remained in fairly constant relation with the palate (fig. 11).

Upper incisor — sc. In the *sc* mice, the upper incisors showed at no time the mature relationship to premaxilla and maxilla attained in the control animals (fig. 4). They failed to grow back from the premaxilla into the maxilla, but remained located anterior to the premaxillo-maxillary suture and their socket was formed only by the premaxilla (figs. 6, 8, 10). After the 12th day, the intra-alveolar portion of the incisor was less than half the length of the control.

This abnormal development could be recognized at birth or one day after birth, when the upper incisor was still entirely surrounded by the premaxilla (fig. 8). The latter was not perforated, nor was the epithelial diaphragm in contact with the premaxillo-maxillary suture, as it was in the normal mice. The change in position of the epithelial diaphragm did not take place. Even with advanced age it was inclined posteriorly and upwards rather than horizontally (fig. 12). At 50 days of age, the dentin near the basal end showed folding, thereby giving the dentino-enamel junction a wavy appearance (fig. 13). In some areas no enamel was formed, and the degenerated ameloblasts were next to the dentin.

Resorption and apposition pattern of upper alveolar bone. In the *control mice,* the premaxillary parts of the sockets of the upper incisors showed a constant pattern of apposition and resorption (fig. 3). On the concave side, the periodontal surface of the premaxilla showed bone apposition which was especially active in the incisal and the basal areas. On the convex side anterior to the premaxillary-maxillary suture, the alveolar bone facing the tooth showed resorption.

The fundic bone, which in the control animals is formed entirely by the maxilla, showed active resorption. The part of the maxilla, however, which formed the alveolar bone on the convex side of the tooth showed varying conditions. In some cases bone apposition, in others resorption, and in still others inactivity was seen.

In the *sc* mice (fig. 4), the pattern of bone apposition and resorption in the alveolar bone proper was essentially the same as in the normal. However, since the entire alveolar bone was furnished by the premaxilla, the various areas of bone apposition and resorption were all found in the premaxilla.

Lower incisor — control. From 17 days in utero on, the basal end of the incisor moved backward in the mandible. In 17-day-old embryos, it was below and anterior to the first molar tooth germ, and at birth was directly below the first lower molar tooth germ (fig. 14). At one day of age, the odontogenic epithelium on the convex side of the incisor had passed the first molar tooth germ. At three days, the entire basal end had passed it, and the pulpal opening was under the second molar germ. At 6 days, the odontogenic epithelium was situated under the germ of the third molar. This backward growth continued until the lower incisor was well past the third molar tooth (fig. 16). In the mouse 30 days old, it was located in the ramus of the mandible.

The fundic bone of the lower incisor was inclined backwards and slightly downwards at birth. At 3 to 4 days it was almost horizontal and maintained that position during the period of observation.[1]

The epithelial diaphragm of the basal end of the incisor was inclined backwards and slightly downwards at birth, and the pulpal opening upwards and slightly backwards (fig. 14). These positions were maintained during postnatal development.

Lower incisor — sc. In *sc* mice, at 17 days in utero, the basal end of the lower incisor occupied the same position as in the controls. At birth, however, it was still below and somewhat anterior to the lower first molar tooth germ (fig. 15). At two days of age it was located under the most anterior part of the first molar germ; at 12 and 15 days, under about the middle of it (fig. 17); and at 20 days of age it was under the posterior margin of the lower first molar crown. No further backward shift beyond this position was seen in older animals.

The fundic bone was inclined downward and slightly backward, or almost in a vertical plane from 4 up to 50 days of life (fig. 17). It never reached or even approached the horizontal plane which the fundic bone of the control lower incisor had almost attained at 3 to 4 days of age. The epithelial diaphragm was either directed downwards with a slight backward inclination or was in a vertical plane. The pulpal foramen opened directly backwards, or backwards with a slight upward inclination (figs. 15, 17).

Resorption and apposition pattern of lower alveolar bone — controls. As in the case of the upper incisors, the alveolar bone on the convex side of the tooth, and the fundic bone, showed bone resorption in all investigated stages. Multinucleated giant cells (osteoclasts) could be seen in large numbers in this area. Opposite this area, on the lower periosteal surface of the mandible, there was very active bone apposition. Rows of osteoblasts were seen.

---

[1] The oldest control animal examined histologically was 30 days old.

The alveolar bone on the concave side of the tooth, anterior to the odontogenic epithelium, showed varying conditions. In some cases bone apposition, and in others bone resorption was seen.

In *sc* mice the pattern of bone apposition and resorption around the lower incisor was similar.

Molars

The development and eruption of the *sc* molars was retarded and their shapes distorted. Whereas in control animals the crown of the first molars was completed at about 6 days, and that of the second at 8 days of age, the *sc* first and and second molar crowns were completed only on the 10th day. First and second molars of the controls erupted around the 15th and 18th day respectively, and those of the *sc* mice between the 20th and 30th day.

The shape of the crowns was fairly normal, but their size was smaller in the *sc* animals (figs. 18, 19). Root formation was more severely disturbed than crown formation. Roentgenograms showed the earliest suggestion of root formation in the first molars of the controls on the 12th day, but in the *sc* mice not until the 20th day. Both roentgenograms and histologic sections showed that the *sc* roots were shortened, narrower and with irregular outlines (fig. 20). The interradicular spaces were narrower, and the alveolar bone lower than in the controls (compare figs. 18 and 19).

The third molars of the *sc* mice were more severely affected than the first and second. They were frequently impacted (fig. 21). In some cases, the roots coalesced and were bent to an acute angle with the axis of the crowns.

DISCUSSION

In the following, an attempt will be made to find a common denominator for the various aberrations in skull and dentition of the *sc* mice. Furthermore, the developmental anomalies seen in these animals will be compared with changes of known endocrine or dietary origin, in an attempt to throw some light on the mechanism by which the *sc* mutation produces its genetic effects.

Pathogenesis of the malocclusion in sc mice

The most striking gross deviations of the *sc* skull were antero-posterior shortening of the mandible, lack of occlusion and failure of backward growth of the incisors, and lack of occlusion, impaction and stunting of the molars. Tentatively, these changes can be explained as the consequence of retarded growth of the mandibular condyle, of the maxillary sutures, and of the connective tissue of the dental pulp.

Effects of deficient growth of the condylar cartilage. The condylar cartilage is the main center for the antero-posterior as well as the vertical growth of the mandible. Retardation of condylar growth in the *sc* mice led to a reduction in the antero-posterior dimension and in the height of the mandible. The antero-posterior shortening, in turn, prevented normal occlusion between upper and lower incisors. This resulted in lack of attrition and excessive growth of their extra-alveolar parts. The deficient downward growth of the mandible, on the other hand, prevented the normal eruption of the lower molars which is dependent on the lowering of the mandible associated with its increase in height.

Effects of deficient growth in sutures. The growth at the premaxillo-maxillary and maxillo-pterygoid sutures contributes to the antero-posterior lengthening of the upper maxillary complex. Normal growth rates at these sites are necessary for the normal size of the maxilla on which positioning and eruption of the upper molars depend. Since the third molars are the last teeth to erupt, their eruption and positioning will be most severely affected by deficient growth at the sutures. In the *sc* mice, growth in both the premaxillo-maxillary and the maxillo-pterygoid sutures was retarded, as evidenced by reduced proliferation and differentiation of the sutural connective tissue and the resulting deficient interdigitation of the bony ridges at the sutures.

Effect of deficient growth of pulpal connective tissue. The backward growth of the basal end of the incisors from the time when the oral epithelium invaginates to the time when the teeth erupt seems to depend on the growth of the connective tissue of the pulp. At about the time of eruption, the connective tissue between the basal ends of the incisors and the alveolar bone differentiates into the hammock ligament (Sicher), which prevents further backward growth of the incisors. In the *sc* mice, the pulpal proliferation is evidently severely depressed. The reduction in pulpal growth is even more severe than that of the premaxillo-maxillary suture. Thus the upper incisor was prevented from growing into the maxilla and remained restricted to the premaxilla. Once the tooth came into function — though reduced by the lack of proper relations between upper and lower incisors — the development of the hammock ligament would have prevented even a late backward growth of the incisors. The failure of the lower incisors to reach the ramus of the mandible caused the ramus to be disproportionately smaller than the anterior part of the body and accounts for the notch seen in the lower border of the *sc* mandible.

Comparison of effects on sutural connective tissue, pulp and cartilage. The growth of condylar cartilage, of the sutural connective tissue of the maxillary complex, and of the dental pulp is retarded in the *sc* mouse, but this effect is quantitatively different in the three sites mentioned.

It was shown above that the depression of growth is more severe in the connective tissue of the pulp than in that of the maxillary sutures. It has also been noted that the *sc* mandible is short, not only as compared with the control mandible, but is also short for the *sc* skull. Figures 5 and 6 clear this point. If the growth of the connective tissue of the sutures and that of the condylar cartilage were disturbed to the same degree, one would have expected a shorter mandible, but one which was "normal" for the *sc* skull. It follows that the *sc* mutation affects the cartilage more severely than the connective tissue, thus leading to a disharmonious stunting of the masticatory mechanism.

Weinmann and Sicher ('50) have pointed out the fundamental difference between the growth processes in the upper and lower jaws; in the former, sutural connective tissue, and in the latter cartilage is the main source of growth. They have suggested that this might be an important factor in the often seen facial and dental disharmonies in man. The present findings further emphasize this often overlooked factor.

That the same genetic or environmental factors inhibit growth of different types of connective tissue, e.g., the pulpal connective tissue and the sutural connective tissue, to a different degree might be another instance of the frequent observation that fast-growing tissues or organs suffer more than those which grow more slowly.

Nutritional and hormonal deficiencies

A comparison of changes produced experimentally with the aberrations caused by the *sc* mutation might point the way to an analysis of the mechanism of genetic action. However, experimental observations suitable for such a comparison are scarce, especially since only a few experiments were performed on animals in utero.

Warkany and Nelson, and Warkany, Nelson and Schraffenberger ('40, '41, '42, '43) showed that about one-third of the offspring of female rats fed on a deficient diet during gestation (Steenbeck and Black's rachitogenic diet supplemented with viosterol) showed multiple skeletal anomalies. Among these were shortening of long bones, shortening of the mandible, fusion of ribs, various deformities of the sternum, scapula, maxilla, and cleft palate. Tibia and mandible were most often affected. Warkany and Schraffenberger later showed ('44) that these congenital deformities could be completely prevented by supplementing the maternal diet with riboflavin. From these experiments they concluded that "the formation of the membranous skeleton which precedes the cartilaginous as well as the osseous skeleton is inhibited by riboflavin deficiency."

Congenital malformations due to maternal deficiency of vitamin A have been described by various authors. Zilva, Golding, Drummond and Coward ('21) observed that sows fed a diet deficient in vitamin A produced pigs in which some limbs were completely missing. Hale ('35, '37) described harelip, cleft palate, accessory ears and abnormal positions of the kidneys in pigs born to sows fed on a vitamin A deficient diet.

Shortness of the limbs and shortness of the antero-posterior axis of the skull was found by Byerly and co-workers ('35) in chick embryos if the mother had been fed a deficient diet. Lyons and Insko ('37) observed shortness of long bones in chick embryos produced by manganese deficiency in the diet of the hen.

All these experiments indicate that maternal dietary deficiency may lead to congenital defects. The anomalies produced by deficiency of riboflavin somewhat resemble those seen in the *sc* mice.

Anomalies of the dentition have been given closer attention in experimental work dealing with hormonal deficiencies.

Ziskin, Salmon and Applebaum ('40) have shown that in rats thyroparathryroidectomized at birth, but receiving parathyroid extract there was shortness of the mandible, retarded development of molar roots, and retarded eruption. Karnofsky and Cronkite ('39) reported that administration of thyroxine greatly accelerated the eruption of the rat incisor.

Schour and Van Dyke ('32, '34) reported retarded eruption and shortened roots of the molars and retarded eruption and folding of the basal ends of incisors of rats hypophysectomized between the ages of 36 and 64 days. Beck, Collins, Simpson and Evans ('46) have reported very similar findings. In hypophysectomy (Schour and Van Dyke, '32, '34) the retardation of the connective tissue proliferation of the dental pulp is manifested in teeth as retardation or arrest of eruption and the subsequent folding of the basal end of the incisor and the shortness of the molar root (Sicher, '42). In both experiments, rats were hypophysectomized at an age when the development of molars was well advanced, when the incisors had grown backwards into the maxilla and mandibular ramus, and had erupted. By comparing the hypophysectomized rats with the *sc* mice, it may be assumed that earlier hypophysectomy might lead to aberrations resembling more closely the anomalies seen in the *sc* mutation.

SUMMARY AND CONCLUSION

The present study is based on anatomic, radiographic and microscopic investigations of the skulls of 58 CSH Bagg Albino strain of mice, of which 34 were screw tail *(sc)* and 24 litter mate controls.

The *sc* skulls were smaller than the controls. They showed antero-posterior shortening of the mandible, lack of occlusion of the incisors and lack of occlusion, stunting and impaction of the molars.

Eruption of the incisors and molars was slightly delayed. The basal ends of upper and lower incisors failed to assume the normal position in maxilla and mandibular ramus. In late stages they showed folding of their basal ends. The molars did not erupt as far and as fast as those in the control animals; their roots were stunted, and the third molar was frequently impacted.

Premaxillo-maxillary and maxillo-pterygoid sutures showed lack of differentiation of sutural connective tissue into the normal layers, and the failure or defective development of sutural interdigitation. The cranial vault instead of the normal two bony plates consisted only of a single plate of bone.

In the fronto-parietal suture, the parietal bone overlapped the frontal bone.

Growth of the cartilage in the mandibular condyle was greatly reduced. The cartilaginous condyle of *sc* mice was about one-half the thickness of the normal. The cartilaginous growth center showed all the layers normally present, but each layer was reduced in thickness.

The morphologic deviations of the *sc* skulls were ascribed to a retardation of connective tissue growth in the dental pulp, sutures, and the condyle.

Quantitatively, the *sc* mutation affected the cartilage growth more than the connective tissue growth, thus leading not only to a stunting of the masticatory mechanism but also to a disharmony between the mandible and the maxillary complex.

ACKNOWLEDGMENTS

The authors wish to express their thanks to Dr. Harry Sicher, Professor of Anatomy, Loyola University School of Dentistry, for his constructive criticism and valuable suggestions, and their gratitude to Mrs. Julia Meyer for her editorial help and to Mrs. Helen Kutuzov for excellent technical assistance.

LITERATURE CITED

BECKS, H., D. A. COLLINS, M. E. SIMPSON AND H. M. EVANS 1946 Changes in the central incisor of hypophysectomized female rats after different post-operative periods. Arch. Path., *41:* 457–475.

BRYSON, V. 1945 Development of the sternum in screw tail mice. Anat. Rec., *91* (2): 119–137.

BYERLY, T. C., H. W. TITUS, N. R. ELLIS AND W. LANDAUER 1935 A new nutritional disease of chick embryo. Proc. Soc. Exp. Biol. and Med., *32:* 1542–1546.

HALE, F. 1935 The relation of Vitamin A to anophthalmos in pigs. Am. J. Ophth., *18:* 1087–1093.

——— 1937 Relation of maternal Vitamin A deficiency to microphthalmia in pigs. Texas State J. Med., *33:* 228–231.

KARNOFSKY, D., AND E. P. CRONKITE 1939 Effect of thyroxine on eruption of teeth in newborn rats. Proc. Soc. Exp. Biol. and Med., *40:* 568–570.

LANNES, T., AND E. C. MACDOWELL 1941 Screw-tail, a new mouse mutation. Rec. of Genetics Soc. Am., *10:* 151–152.

LYONS, M., AND W. M. INSKO, JR. 1937 Chondrodystrophy in chick embryo produced by a mineral deficiency in the diet of the hen. Science, *86:* 328.

MACDOWELL, E. C., J. S. POTTER, T. LAANES AND E. N. WARD 1942 The manifold effects of the screw tail mouse mutation. J. Heredity, *33:* 439–449.

SCHOUR, I., AND H. B. VAN DYKE 1932 Changes in the teeth following hypophysectomy. I. Changes in the incisor of the white rat. Am. J. Anat., *50:* 397–433.

——— 1934 Changes in the teeth following hypophysectomy. II. Changes in the molars of the white rat. J. Dent. Res., *14:* 69–91.

SICHER, H. 1942 Tooth eruption. The axial movement of continuously erupting teeth. J. Dent. Res., *21:* 201–210.

——— 1950 Personal communication.

WARKANY, J., AND R. C. NELSON 1940 Appearance of skeletal abnormalities in the offspring of rats reared on a deficient diet. Science, *92:* 383–384.

——— 1941 Skeletal abnormality in the offspring of rats reared on deficient diet. Anat. Rec., *79:* 83–100.

——— 1942 Skeletal abnormality induced in rats by maternal nutritional deficiency — histologic studies. Arch. Path., *34:* 375–384.

WARKANY, J., R. C. NELSON AND E. SCHRAFFENBERGER 1943 Congenital malformations induced in rats by maternal nutritional deficiency. III. The malformations of extremities. J. Bone and Joint Surgery, *25:* 261.

WARKANY, J., AND E. SCHRAFFENBERGER 1944 Congenital malformations induced in rats by maternal nutritional deficiency. VI. The preventive factor. J. Nutrition, *27:* 477–484.

WEINMANN, J. P., AND H. SICHER 1950 Personal communication.

ZILVA, S. S., J. GOLDING, J. C. DRUMMOND AND K. H. COWARD 1921 The relation of the fat soluble factor to rickets and growth in pigs. Biochem. J., *15:* 427–437.

ZISKIN, D. E., T. N. SALMON AND E. APPLEBAUM 1940 The effect of thyroparathyroidectomy at birth and at 7 days on dental and skeletal development of rats. J. Dent. Res., *19:* 93.

PLATES

KEY TO ILLUSTRATIONS

AB, alveolar bone
B, brain
D, dentin
E, enamel space
ED, epithelial diaphragm
EO, enamel organ
FB, fundic bone
M, maxilla
M_1, first molar
M_2, second molar
M_3, third molar
NB, nasal bone
NC, nasal cavity
NL, nasolacrimal duct
O, oral epithelium
P, pulp
PF, pulpal foramen
PM, premaxilla
PMS premaxillo-maxillary suture
T, tongue

PLATE 1

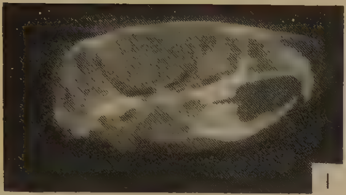
EXPLANATION OF FIGURES

1, 2 Roentgenograms of the skulls of a control (fig. 1) and an *sc* mouse (fig. 2), 80 days of age. Note the anterior position of incisors, shorter roots of the molars, impaction of third molars, distortion of the shape of the mandible and the single plate of bone of the cranial vault in figure 2.

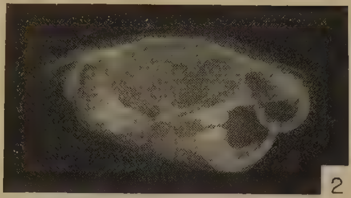
3, 4 Diagrams of the upper incisors and their supporting bones in the control (fig. 3) and *sc* mouse (fig. 4) about 30 days of age. Note that both premaxilla and maxilla contribute to the alveolar socket of the control incisor while only the premaxilla surrounds the *sc* incisor. +, apposition; —, resorption.

5 Photomicrograph showing the upper incisor and its surrounding structures of the control mouse 10 days of age. Note that the epithelial diaphragm and the fundic bone are horizontally placed and the premaxillo-maxillary suture is located anterior and inferior to the basal end of the incisor. Also note the relation between the tongue and the incisal tip. H & E, × 23.

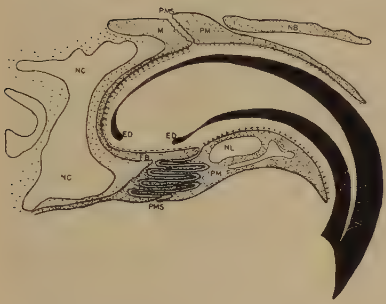
6 Photomicrograph showing the upper incisor and its surrrounding structures of an *sc* mouse 10 days of age. Note that it is smaller in size, its epithelial diaphragm and fundic bone are vertically placed, and that the premaxillo-maxillary suture is located posterior and inferior to its basal end. Compare the relationship of the tongue and the incisal tip in figures 5 and 6. H. & E, × 23.



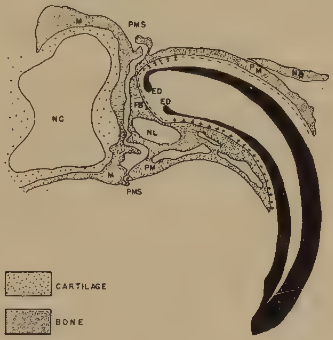
1



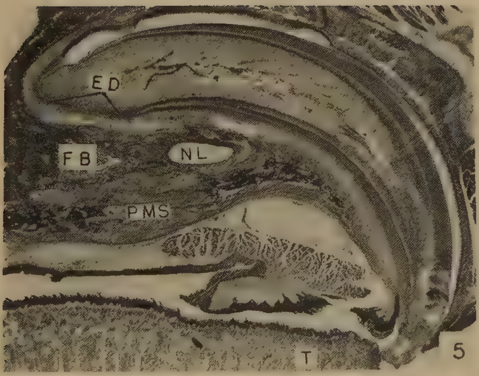
2


PMS
M
PM
NB
NC
ED
ED
NL
FB
PM
NC
PMS

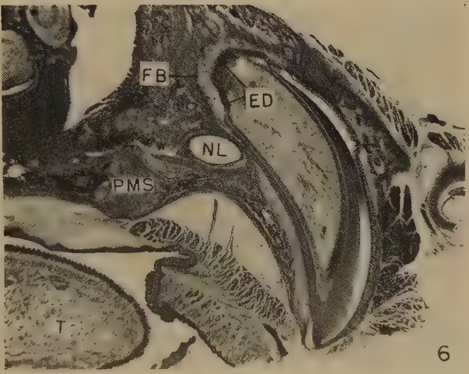
3


M
PMS
PM
NB
ED
ED
FB
NC
NL
PM
M
PMS
CARTILAGE
BONE

4


ED
FB
NL
PMS
T

5


FB
ED
NL
PMS
T

6

PLATE 2

EXPLANATION OF FIGURES

7 Photomicrograph showing the basal end of the upper incisor (area shown in the insert) of a control mouse one day of age. The premaxilla in the area S has been perforated by the backward-growing basal end and the maxillary bone forms that part of the alveolus of the tooth. The epithelial diaphragm is almost vertically placed and the pulpal foramen opens directly backward. H & E, × 82.

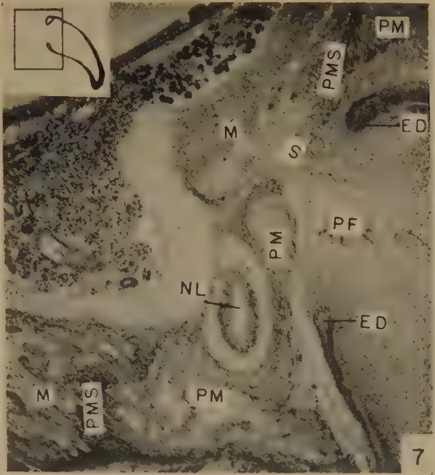
8 Photomicrograph showing the basal end of the upper incisor (area shown in the insert) of an *sc* mouse one day of age. The basal end of the incisor is completely surrounded by the premaxilla and both the fundic bone and the epithelial diaphragm are vertically placed. H & E, × 82.

9 Photomicrograph showing the basal end of the upper incisor (area shown in the insert) of a control mouse 4 days of age. Note that the basal end of the incisor has grown into the maxilla and that the maxilla forms most of the fundic bone. The premaxillo-maxillary suture lies below the pulpal foramen. The epithelial diaphragm and the fundic bone are inclined backward and upward. H & E, × 82.

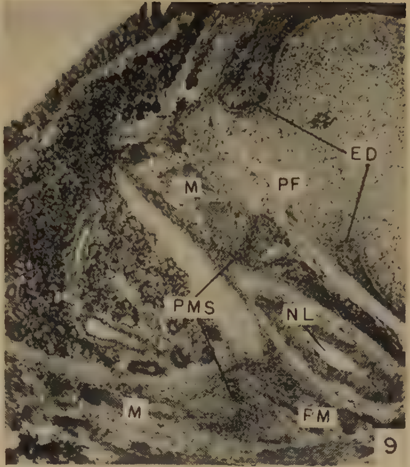
10 Photomicrograph showing the basal end of the upper incisor (area shown in the insert) of an *sc* mouse 4 days of age. Note that the entire basal end of the incisor lies anterior to the premaxillo-maxillary suture and the fundic bone and the epithelial diaphragm are vertically inclined. H & E, × 82.

11 Photomicrograph showing the basal end of the incisor (area shown in the insert) of a control mouse 20 days of age. The epithelial diaphragm and fundic bone are horizontally placed and the pulpal foramen opens downward. H & E, × 61.

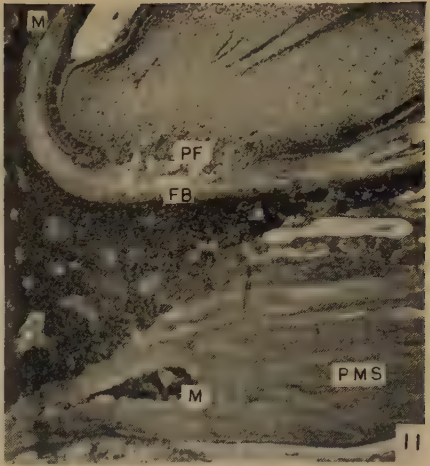
12 Photomicrograph showing the basal end of the incisor (area shown in insert) of an *sc* mouse 20 days old. The incisor lies anterior to the premaxillo-maxillary suture. The epithelial diaphragm and the fundic bone are inclined backward and upward. H & E, × 61.


PM
PMS
M
S
ED
PF
PM
NL
ED
M
PMS
PM

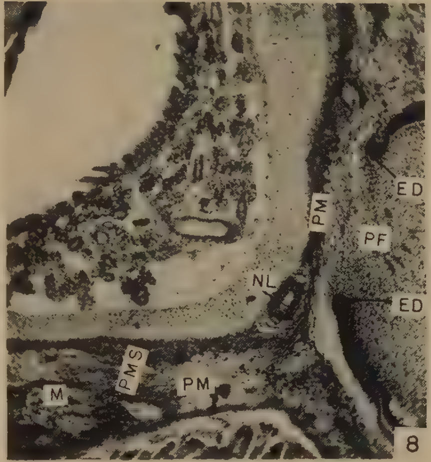
7


ED
M
PF
PMS
NL
M
FM

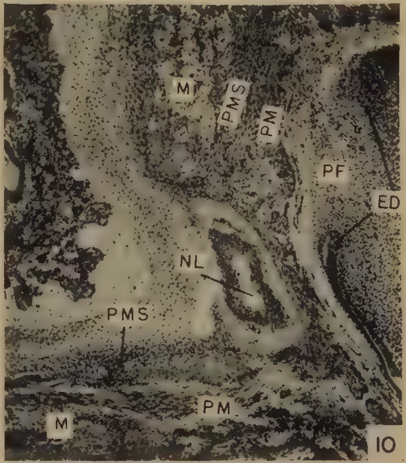
9


M
PF
FB
PMS
M

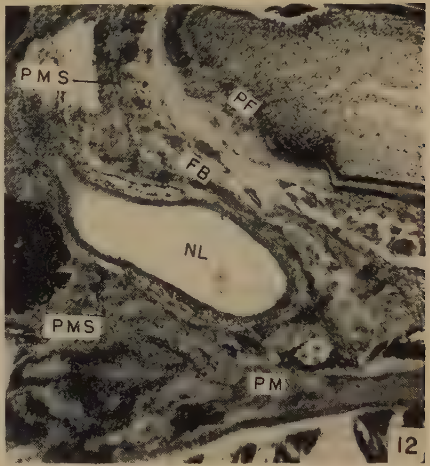
11


ED
PM
PF
NL
ED
PMS
M
PM

8


M
PMS
PM
PF
ED
NL
PMS
M
PM

10


PMS
PF
FB
NL
PMS
PM

12

PLATE 3

EXPLANATION OF FIGURES

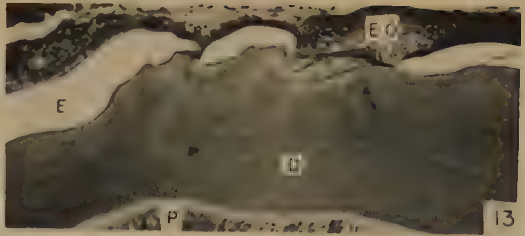
13 Photomicrograph showing a part of the convex side of the upper incisor of an *sc* mouse 50 days of age. Note the folding of the dento-enamel junction and absence of enamel at two areas. H & E, × 120.

14 Photomicrograph showing the basal end of the lower incisor of a control mouse at birth. The basal end lies below the first molar tooth germ and the pulpal foramen is directed upward and slightly backward. H & E, × 42.

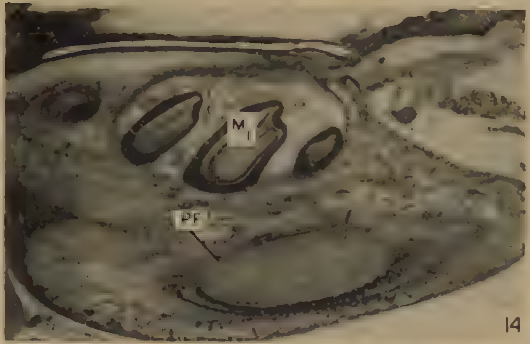
15 Photomicrograph showing the basal end of the lower incisor of an *sc* mouse at birth. The basal end lies below and anterior to the first molar tooth germ and the pulpal foramen opens directly backward. H & E, × 42.

16 Photomicrograph showing the basal part of the lower incisor of a control mouse 15 days of age. Note that the basal end is posterior to the molars. H & E, × 23.

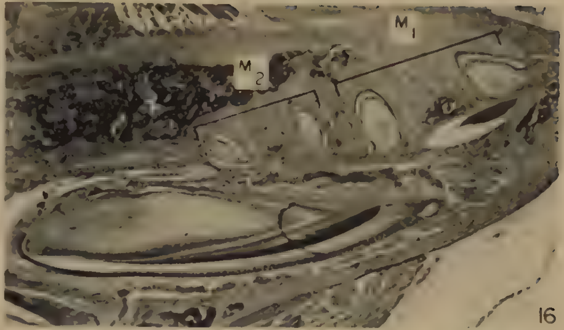
17 Photomicrograph showing the basal part of the lower incisor of an *sc* mouse 15 days of age. Note that the basal end is still under the first molar and has failed to grow backward.The pulpal foramen opens directly backward. H & E, × 23



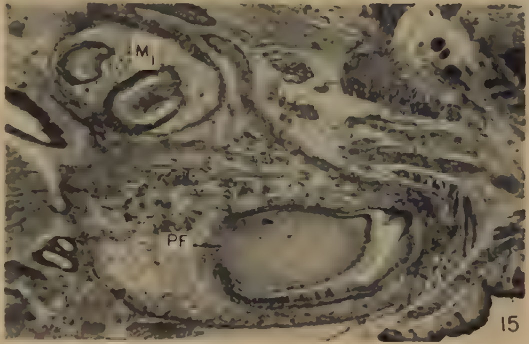
13



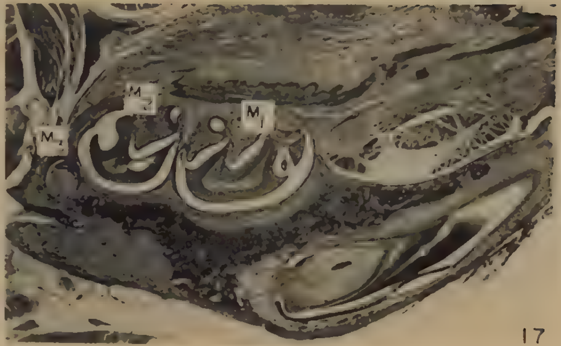
14



16



15



17

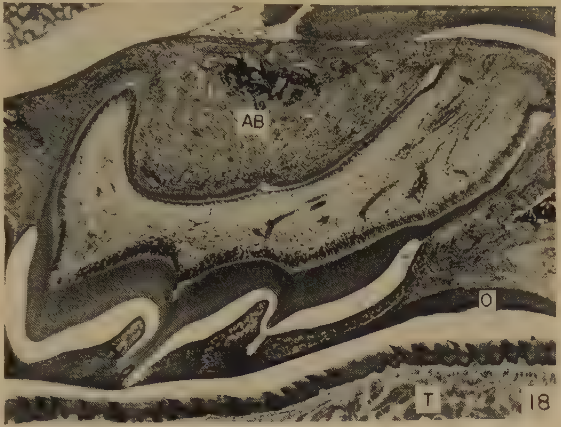
PLATE 4

EXPLANATION OF FIGURES

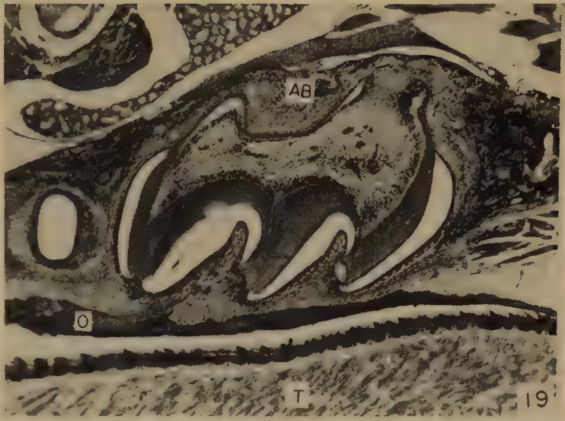
18, 19 Photomicrographs showing central sections through the mesial roots of upper first molars of a control (fig. 18) and *sc* mouse (fig. 19) 15 days of age. Note that the *sc* molar is smaller, its roots are stunted and its alveolar process is much reduced in height as compared with the control. H & E, × 46.

20 Photomicrograph showing the upper first molar of an *sc* mouse 30 days of age. Note the distorted outline of the mesial root. H & E, × 46.

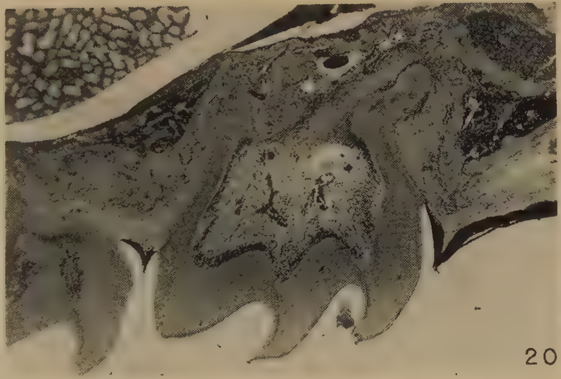
21 Photomicrograph showing the upper third molar of an *sc* mouse 50 days of age. The shape of the crown is distorted and it is impacted. H & E, × 63.



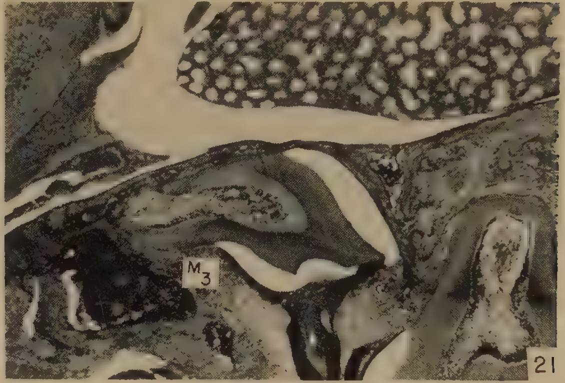
18



19



20



21

PLATE 5

EXPLANATION OF FIGURES

22, 23 Photomicrographs showing the premaxillo-maxillary suture of a control (fig. 22) and *sc* mouse (fig. 23) at one day of age. Note that in the *sc* suture the interdigitation of the two bones is missing and the bones are smaller in thickness. H & E, × 110.

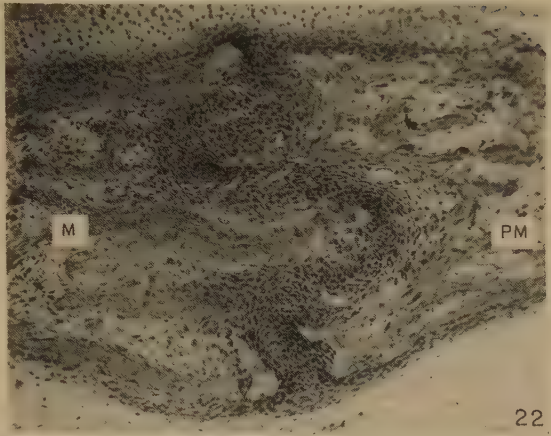
24, 25 Photomicrographs showing the premaxillo-maxillary suture of a control (fig. 24) and an *sc* mouse (fig. 25) 10 days of age. Note the complete absence of interdigitation of bones in the latter. H & E, × 52.

26 Photomicrograph showing the parietal bone of a control mouse 30 days of age. Note two plates of bone enclosing haemopoietic bone marrow. H & E, × 200.

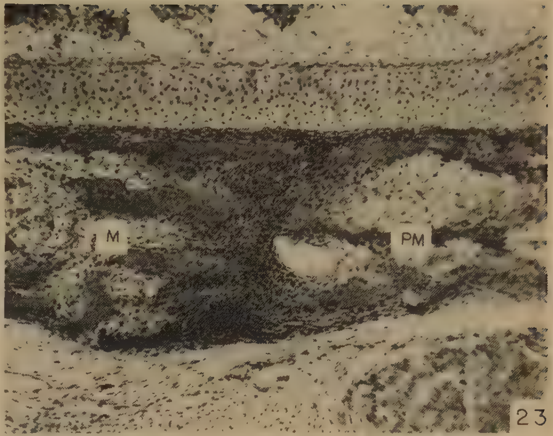
27 Photomicrograph showing the parietal bone of an *sc* mouse 30 days of age. Note the single plate of bone and absence of haemopoietic bone marrow. H & E, × 200.

SKULL AND DENTITION OF *sc* MICE
BHASKAR, SCHOUR, MACDOWELL AND WEINMANN

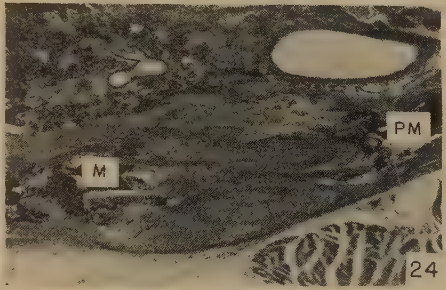
PLATE 5



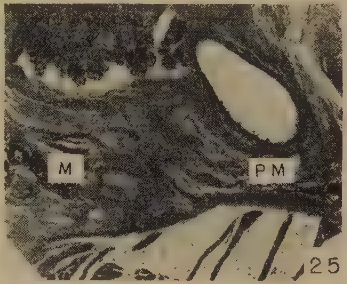
22



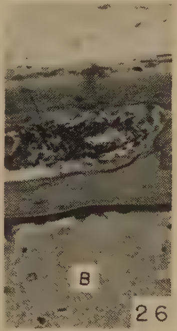
23



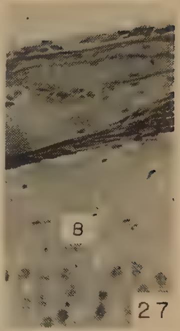
24



25



26



27

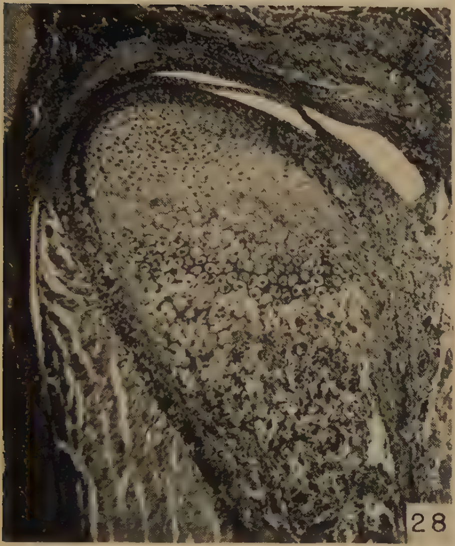
PLATE 6

EXPLANATION OF FIGURES

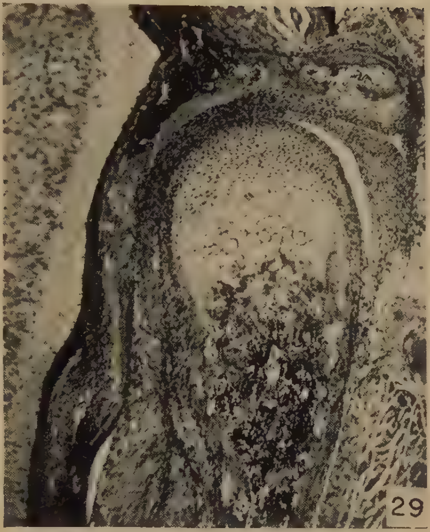
28, 29 Photomicrographs showing the mandibular condyle of a control (fig. 28) and an *sc* mouse (fig. 29) 6 days of age. Note the reduced thickness of the condylar cartilage and proximity of the red bone marrow to the cartilage front in the *sc* condyle. H & E, × 85.

30 Photomicrograph showing the mandibular condyle of a control mouse 30 days of age. H & E, × 85.

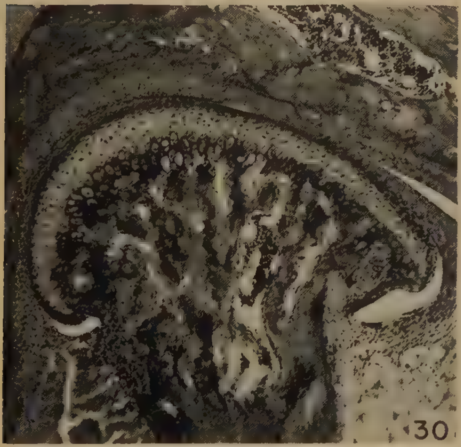
31 Photomicrograph showing the mandibular condyle of an *sc* mouse 30 days of age. H & E, × 85.



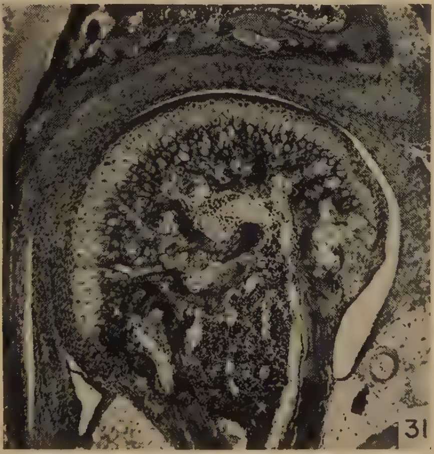
28



29



30



31

THE DEMONSTRATION OF THIOL GROUPS IN CERTAIN TISSUES BY MEANS OF A NEW COLORED SULFHYDRYL REAGENT[1,2]

H. STANLEY BENNETT

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, and the Department of Anatomy,[3] University of Washington, Seattle, Washington

FIVE FIGURES

INTRODUCTION

This paper reports the application of a new red sulfhydryl reagent, 1-(4-chloromercuriphenylazo)-naphthol-2, as a qualitative reagent for the localization of mercaptans in minute tissue and cellular components visible with the light microscope. Evidence for the specificity of the red sulfhydryl reagent is presented, the method of use is cited, and the detection by this reagent of sulfhydryl groups in certain animal tissue components where thiols have not been previously described is reported.

The qualitative localizing observations herein reported were made incidentally during the course of an effort to develop a quantitative analytical cytochemical method for measuring the sulfhydryl content of tissue components too small to be analyzed by conventional microchemical procedures. No systematic effort has been made to explore a great variety of tissues qualitatively with this technique.

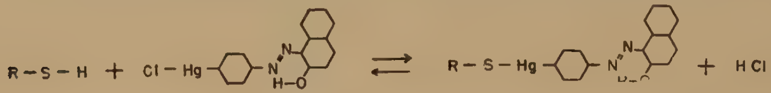
---

[1] This investigation has been supported in part by a grant from the American Cancer Society recommended by the Committee on Growth of the National Research Council.

[2] A preliminary report of some phases of this study has been published in abstract form (Bennett, Anat. Rec., *100:* supplement, pp. 640, 732, 1948).

[3] Present address.

The procedure utilized can be regarded as a modification of the phenyl mercuric chloride sulfhydryl reaction of Hellerman, Perkins, and Clark ('33). It is based on a simple red-colored azo derivative of Hellerman's reagent. This red derivative was synthesized so as to retain the specificity for mercaptans displayed by phenyl mercuric chloride, and to show the added property of behaving as an optical tracer or tag which would delineate by virtue of its color the sites at which it is bound to tissue components by mercaptide linkage. The basic reaction can be represented as follows:



(1)

Conditions are employed wherein the equilibrium is forced far to the right.

METHOD

1 - (4 - chloromercuriphenylazo) - naphthol - 2, hereafter referred to as the red sulfhydryl reagent, abbreviated RSR, was synthesized and recrystallized according to the method of Bennett and Yphantis ('48). The sample used was judged to be reasonably pure, and yielded analyses for carbon, nitrogen, hydrogen, and mercury as cited by Bennett and Yphantis ('48).

Tissue was prepared for reaction according to two different methods:

a. By fixation in 5% trichloracetic acid, followed by washing in distilled water, dehydration through graded alcohols, immersion in n-propanol, or n-butanol, and teasing or fragmentation into small pieces.

b. By freezing of small bits or strips of tissue in isopentane cooled to about — 180°C. in liquid nitrogen, followed by immersion of the frozen tissue into n-propanol or n-butanol at — 20°, without allowing the tissue to thaw. The tissue was then permitted to stay in cold solvent for 10 or 12 days with occasional changes of solvent, but kept continuously below — 20°. During this period the butanol or propanol slowly dissolved away the frozen water in the tissue, and replaced little by little the solid aqueous medium with a liquid solvent one. When dehydration was complete the tissue was teased into small pieces suitable for microscopic study.

Teased tissue fragments prepared by either of the above methods were then placed in a saturated solution of RSR in butanol (about 1.25×10^{-5} M) or in propanol (about 5.6×10^{-6} M), and left in the solution for a sufficient period of time to permit the solute to penetrate and react with available sulfhydryl groups in the tissue. The time required to complete this reaction depended on the solvent, the concentration of the reagent, and the size of the tissue fragments. Teased, single, whole skeletal muscle fibers immersed in saturated RSR in n-propanol would usually react to completion in a few hours or overnight. After completion of the reaction the fragments were washed in several changes of pure solvent, allowing adequate time (one to three hours) in each washing to remove any unbound red reagent in the tissue. The fragments were then run through xylene and mounted on slides in clarite in the usual manner.

Parallel control slides of two types were prepared in each case from equivalent bits of tissue. One set of control slides consisted of tissue prepared as above, but immersed in a solution of phenyl azo β-naphthol instead of in RSR. The other set of controls was made up of tissues likewise treated as above, but desulfhydrylated in some suitable manner before immersion in the red sulfhydryl reagent. Significance was attached to the binding of RSR to tissue only when both types of control preparations showed no bound red reagent.

It was possible to utilize the procedure on sectioned material as well as on teased fragments. Trichloracetic acid fixed or frozen cold alcohol-dried tissue was embedded in paraffin or nitrocellulose, sectioned, and mounted on slides without albumin (as albumin contains mercaptan groups which may confuse a tissue reaction). The sections were then subjected to the action of RSR or the control procedures.

At all steps precautions were taken to insure the purity of the reagents used. Particular care was exercised to avoid traces of heavy metals and sulfur compounds. Trichloracetic acid was purified by distillation under reduced pressure. All organic solvents were selected for purity and redistilled; the wash water was redistilled and protected from contamination. The red sulfhydryl reagent solutions were protected from elevated temperatures and strong light (which can decompose azo compounds).

The specificity of the reaction

The basic reaction of aryl or alkyl mercuric halides with protein and other mercaptans has been studied extensively, and good evidence for specificity has been obtained (Hellerman, Perkins, and Clark, '33; Anson, '41; Singer and Barron, '45; Olcott and Fraenkel-Conrat, '47; and Hughes, '50). However, the application of such compounds to more complicated organized tissue components has not been reported. Moreover, the red sulfhydryl reagent used in this study embodies in its molecule certain groupings not present in the colorless organic mercurials utilized heretofore as sulfhydryl reagents, and hence its specificity must be examined in this light.

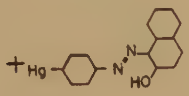
An inspection of the structural formula of the red sulfhydryl reagent calls to mind the possibility that it might bind to tissue components in any of 4 different ways:

(a) Specifically in covalent mercaptide linkage to tissue sulfhydryl groups, as indicated in the chemical reaction (1).

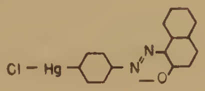
(b) Non-specifically by hydrogen bonding to the azo nitrogen or the oxygen of the molecule by suitable positive hydrogens in tissues, such as those embodied in tissue O-H or N-H groups. The hydrogen of the O-H grouping of the red reagent would not appear likely to engage in hydrogen bond formation with electronegative tissue atoms, as it is probably firmly chelated by intramolecular hydrogen bonding to one of the azo nitrogens, and hence not available for intermolecular hydrogen bonding (Mason, '31; Elkins and Hunter, '35).

(c) Non-specifically by van der Waals forces, acting mainly between the aromatic rings of RSR and tissue components.

(d) Non-specifically by salt linkages, between the



or the



ions and some tissue ion group, such as phosphate. In view of the very low degree of dissociation of the aryl mercuric halides or of orthoazo phenols or azo β-napthols, significant binding by salt linkage would not be expected, and, as is reported subsequently in this paper, has been demonstrated not to occur.

For a discussion of the fundamental nature of the 4 types of linkages, see Pauling ('45).

Successful application of this reagent as a specific cytochemical molecular tag for mercaptans depends, then, on setting up experimental conditions whereby non-specific binding does not occur, or whereby any non-specifically bound reagent can be removed, leaving only the specific mercaptide-bound reagent attached to the tissue. One can hope to accomplish this by using an excess of organic solvents which can compete successfully by mass action effect for any red mercurial which might be lashed to tissue components by hydrogen bonds or van der Waals forces. Butanol and propanol can be expected to behave as such competitive solvents, and would not be expected to compete with tissue thiol groups for specifically bound RSR.

Evidence for the specificity of the method was sought by exploring the following three questions:

1. Does the binding of the red sulfhydryl reagent to tissue depend on the presence in the reagent molecule of the chloromercuri group, and not on some other component?

2. Does the binding of RSR to tissue depend on the presence of intact sulfhydryl groups in the tissue?

3. When bound to tissue, is the red mercurial attached by specific covalent mercaptide linkage, or by non-specific hydrogen bonds, salt linkages or van der Waals forces?

Suitable test objects with which answers to the above three questions could be sought were found in the form of single striated muscle fibers of frogs or rats, teased out in propanol from thin strips of muscle which had previously been frozen and alcohol-dried, or which had been fixed in trichloracetic acid. Skeletal muscle has long been known to be rich in sulfhydryl groups (Hopkins, '21), and the presence of bound RSR in the muscle fiber can be recognized readily either by gross inspection or under the microscope.

An answer to question 1 was sought by placing suitably prepared muscle fibers into a propanol or butanol solution of RSR, and an equivalent control group of fibers into a solution of phenyl azo β-naphthol, which is identical in molecular structure to RSR, save that it lacks the chloromercuri group, having in its place a hydrogen. After immersion for a few hours the fibers were washed in the solvent and examined. Those that had been immersed in the RSR were found to be colored red, whereas those from the phenyl azo β-naphthol solution were colorless, indicating that under these conditions the presence of the chloromercuri group on the molecule was necessary for binding, and that the azo nitrogen and O-H groups and aromatic ring portions of the molecule were not capable of forming stable linkages with any component of muscle tissue.

Question 2 was explored by comparing the binding of RSR to muscle fibers prepared as above and immersed directly in RSR solution to the binding to similar fibers subjected previously to a desulfhydrylating procedure. Desulfhydrylation procedures depending on three different principles were utilized.

(a) By oxidation:

$$\text{R-SH} + \text{H-S-R} \xrightarrow[\text{agents}]{\text{oxidizing}} \text{R-S-S-R} + 2\text{H} \qquad (2)$$

Iodine, or hydrogen peroxide in the presence of ferric ion can be used as oxidizing agents.

(b) By alkylation:

$$\text{R-SH} + \text{I-CH}_2\text{-R}' \longrightarrow \text{R-S-CH}_2\text{-R}' + \text{HI} \qquad (3)$$

Iodoacetic acid or iodoacetamide are suitable alkylating substances.

(c) By mercaptide formation:

$$\text{R-SH} + \left\{\begin{matrix} \text{Cl-Hg-R}' \\ \text{or} \\ \text{I-Hg-R}' \end{matrix}\right. \longrightarrow \text{R-S-Hg-R}' + \left\{\begin{matrix} \text{H Cl} \\ \text{or} \\ \text{HI} \end{matrix}\right. \qquad (4)$$

Phenyl mercuric chloride, tolyl mercuric chloride, and methyl mercuric iodide are suitable mercaptide forming reagents.

Tissue O-H, phosphate, carboxyl, amine, amide, or other groups, which might conceivably bind RSR non-specifically, would not be affected by the above reagents, except that the alkylating reagents can also react with tissue amines.

The following desulfhydrylating solutions were prepared:[4]

SOLUTE	SOLVENT	CONCENTRATION
I_2	n-propanol	0.001 M
H_2O_2 and $Fe\ Cl_3$	iso-propanol or H_2O	1.0 M .001 M
iodoacetamide	n-propanol	0.1 M
iodoacetic acid	n-propanol	0.1 M
phenyl mercuric chloride	n-propanol	0.001 M
tolyl mercuric chloride	n-propanol	0.001 M
methyl mercuric iodide	n-propanol	0.001 M

No significance is attached to the use of iso-propanol in some cases and n-propanol in others. Most of the solutes were also tried in n-butanol solution with identical results.

Fixed or frozen alcohol-dried muscle fibers in propanol or butanol solvent were placed in each of the above solutions for a few hours, rinsed free of solute, and then placed in a solution of RSR at the same time that the untreated fibers were placed in a similar medium. After 3 or 4 hours all fibers were removed from RSR solution, rinsed in solvent, and examined. It was found that all fibers which had been subjected for a sufficient length of time to a desulfhydrylating reagent prior to contact with RSR remained colorless,[5] whereas those placed in RSR without prior treatment with a desulfhydrylating agent were red. These findings were interpreted as evidence that the presence of mercaptan groups in tissues is necessary for binding RSR, and that other tissue groups are not implicated in the reaction.

---

[4] The iodine, hydrogen peroxide, and ferric chloride were analytical grade chemicals. The iodoacetic acid, phenyl mercuric chloride, and tolyl mercuric chloride were obtained from Eastman Kodak Company, Organic Chemical Sales Division. The iodoacetamide was made from Eastman chloroacetamide by the method of Anson ('39). The methyl mercuric iodide was synthesized and supplied by Doctor W. L. Hughes, Jr., to whom I am grateful.

An answer to question 3 was obtained by preparing a large number of muscle fibers which were permitted to react with RSR until maximally red. Samples of these fibers were then placed in a series of fluids or solutions each designed to compete with molecules bound in tissue by a certain type of bond.

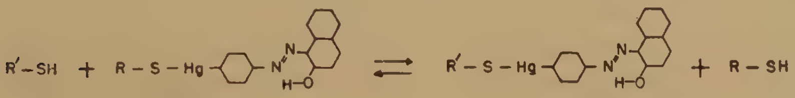
The following fluids were prepared:

SOLUTE	SOLVENT	CONCENTRATION	TO COMPETE WITH MOLECULES BOUND BY
ethanol	H_2O	70%	hydrogen bonds
propanol	H_2O	50%	hydrogen bonds
acetic acid	H_2O	0.1 N	hydrogen bonds salt linkages
Na Cl	H_2O	1.0 M	salt linkages
Ca Cl_2	H_2O	0.1 N	salt linkages
Na OH	H_2O	0.1 N	salt linkages
NH_4OH	H_2O	0.1 N	salt linkages
	toluene	100%	van der Waals forces
	benzene	100%	van der Waals forces
β-mercaptoethanol	H_2O or propanol	0.01 M	covalent mercaptide linkages
cysteine	H_2O	0.01 M	covalent mercaptide linkages
BAL	iso-propanol	0.01 M	covalent mercaptide linkages
glutathione	H_2O	0.01 M	covalent mercaptide linkages
thioglycolic acid	H_2O or propanol	0.01 M	covalent mercaptide linkages
methionine	H_2O or propanol	0.01 M	weak competitor for hydrogen bonds and salt linkages
cystine	H_2O or propanol	saturated solution	weak competitor for hydrogen bonds and salt linkages

---

[5] It was found that when muscle fibers desulfhydrylated with phenyl mercuric chloride were immersed in RSR for a prolonged period such as that of a day or more, the fibers gradually acquired a pale pink color which deepened with time. This is not interpreted as evidence against the specficity of the reaction.

Aliquots of red fibers were exposed to the action of each of the above fluids overnight, and were then rinsed free of reagent, mounted in clarite, and examined. It was found that all fibers retained the red color without noticeable diminution except for those which were immersed in solutions containing a mercaptan. The fibers treated with solutions of β-mercaptoethanol, thioglycolic acid, BAL, cysteine, or glutathione were completely decolorized. This decolorization can be explained according to the following well-known equilibrium reaction involving mercaptides:



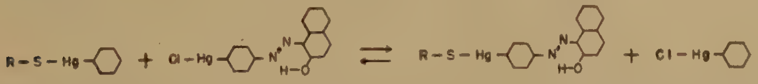
(6)

In the presence of a large excess of competing mercaptan the equilibrium would be shifted greatly to the right by the mass action effect, and the aryl mercurial would be removed from the tissue and transferred virtually completely to the mercaptan in solution.

Muscle fibers once bearing red mercaptides at the former sites of their sulfhydryls, could, after decolorization by a mercaptan, readily be caused to reassume their red color by reimmersing them in a suitable solution of RSR. This sequence of alternate reddening of fibers in RSR, followed by decolorization in a mercaptan solution, was found to be repeatable

---

It is regarded as indicating that when all tissue mercaptan sites are occupied by phenyl mercaptide radicals, a rapid uptake of RSR is prevented so that within a few hours no noticeable amount of RSR is bound. However an equilibrium appears to take place, wherein some of the phenyl mercaptides are replaced by red ones, producing an effect which becomes noticeable in a day or so, and which proceeds until an equilibrium is established wherein the final proportion of sulfhydryl sites occupied by red mercaptide groups depends in part on the relative concentration of the phenyl mercurial as compared to the azo mercurial. This equilibrium might be represented thus:



(5)

This equilibrium has not been studied in detail, and a similar one has not been found described in the literature.

over at least 5 or 6 cycles. These observations are interpreted as evidence that removal of the red mercaptide from muscle by a thiol restores the muscle sulfhydryl groups at their original sites, in accordance with reaction (6).

It is significant that methionine and cystine did not decolorize the muscle fibers, although these reagents contain sulfur. They are not mercaptans and should not be able to remove bound mercaptides. They would be expected to compete somewhat for substance bound ionically or by hydrogen bonds, by virtue of their amino and carboxyl groups.

These data are interpreted as evidence that the red mercurial is bound to tissue by stable specific covalent mercaptide linkages, and not by non-specific hydrogen bonds, salt linkages, or van der Waals forces. These basic tests were applied likewise to nerve, epidermis, crystalline lens, and numerous other tissues, with results similar to those obtained with muscle.

Thus the answers to questions 1, 2, and 3 were found by experiment to be satisfactory, and to support the view that the basic reaction utilized in this study is specific for thiol groups, and that the sites of binding of the red mercurial, 1 - (4 - chloromercuriphenylazo) - naphthol - 2 to tissue under proper conditions can be interpreted as direct evidence for the presence of sulfhydryl groups at those sites.

OBSERVATIONS

The observations reported herein were all made on teased tissue fragments, and were controlled as outlined under Methods.

The red mercurial was observed to be bound to striated skeletal and cardiac muscle of frogs, rats, mice, and lizards, and to the flight muscles of flies and the giant silkworm moth, *Platysamia cecropia.* It was found not to color connective tissue noticeably. The contrast between the strong sulfhydryl reaction in muscle and its absence in collagenous tissue is shown in figure 1. It was likewise shown to color liver and kidney tissue, erythrocytes, epidermis, cortical portion of crystalline lens, pancreas, and other glands. Evidence for the presence of sulfhydryl in cellular organs and its absence in extracellular collagenous connective tissue has long been known, and these observations are in conformity with numerous reports in the literature (for references, see Discussion).

Teased fragments from the cortical portion of the crystalline lens of frogs and mice where sulfhydryl groups have been found previously (Rosner, Farmer, and Bellows, '38; Hermann and Moses, '45) showed, when examined under the microscope, that the sulfhydryl was not evenly distributed throughout the cortical lens fibers, but was concentrated at the surface or between the fiber cells and in thin strands which weaved an irregular pattern within or amongst the cells (fig. 4).

RSR has been found to react with tissues where sulfhydryl has not previously been reported. Amongst them are the following:

Capillary endothelium. Capillaries located amongst teased skeletal muscle fibers of frog and rat were observed with the microscope to show a distinct but pale pink color after treatment with RSR (fig. 5), but were colorless in control preparations. This color appeared to be in the endothelium.

Nerve axoplasm and ganglion cell bodies. Nerves amongst teased striated muscle fibers of frogs, rats and *Platysamia cecropia* likewise assumed pink color when immersed in RSR, and remained colorless in the control preparations. Under the microscope this color was observed to be in the axis cylinder (fig. 3). That the myelin sheath was free from bound mercaptide was not determined with certainty and this evidence for sulfhydryl in nerve axoplasm should not be interpreted as indicating its absence in the myelin sheath. The same sulfhydryl reaction was found in the axis cylinders of the sciatic and sympathetic nerves of frogs and rats, in the ganglion cell bodies of sympathetic and dorsal root ganglia and in the retina in these animals, and in the giant axon of the squid, where it was observed in the axoplasm.

Retinal rods. Frozen alcohol-dried retinae of frogs and mice were placed in butanol solutions of RSR and found to assume a pink color. Upon examination with the microscope, the retinal rods were observed to be distinctly pink (fig. 2). Control preparations were colorless.

Insect trachea. Trachea found amongst teased flight and leg muscle fibers of the giant silkworm moth, *Platysamia cecropia,* were found to be pink after immersion in RSR solution, and colorless in control specimens. It was not ascertained just where in the trachea wall the mercaptans were localized.

DISCUSSION

Tissues of animals and plants have been explored extensively in search of mercaptans by means of the nitroprusside reaction (Gola, '02; Buffa, '04; Heffter, '08; Arnold, '11; Hopkins, '21; Walker, '25; Joyet-Lavergne, '27a, b, '28; Giroud, '28, '29, '30, '31; Giroud and Bulliard, '28, '29, '30; Hammett, '33a, b; Junqueira, Fajer, Rabinovitch and Frankenthal, '49) and by the ferricyanide reaction (Chèvremont and Fréderic, '43).

The present study extends somewhat the list of bodily structures in which mercaptans have been demonstrated, and introduces a cytochemical procedure for demonstrating sulfhydryl which, although rather insensitive, stands up well under the tests for specificity more rigorous and extensive than those which have been applied to other methods. The nature of the sulfhydryl groups entering into the reaction has not been studied in detail. One can presume that most or all of the glutathione is removed in the trichloracetic acid-fixed tissues, and that protein sulfhydryl accounts for most of the reaction in such material. Glutathione is very nearly insoluble in propanol and butanol. Hence, in frozen preparations dried in these solvents, glutathione may account for part of the RSR bound. In frozen and in chemically fixed material, some protein denaturation can be assumed to occur, and hence so-called ''masked'' as well as ''free' sulfhydryl groups probably enter into the reaction.

Buffa ('04) and Heffter ('08) obtained a strong nitroprusside reaction from brain tissue. Buffa reported a distinct reaction in the white matter of the brain, Süllman and Schmid ('42) and Kodomari ('40) found glutathione in the retina, but no reports have been found of a sulfhydryl reaction located definitively in the axoplasm or in the nerve cell bodies or specifically in retinal rods.[6] The demonstration of mercaptans in the axis cylinder suggests that it might be of interest to explore the role of these groups in the neuritis of mercury and lead poisoning. Moreover, it calls attention to an additional similarity of nerve to muscle, which likewise contains sulfhydryl groups in its cellular substance.

The finding of mercaptan groups in capillary endothelium highlights the observation of Danielli, Danielli, and Mitchell ('47) that arsenicals increase capillary permeability, and that intravenous adminstration of a suitable non-toxic competing thiol compound can be used as a preventive or treatment. The present observations provide evidence for definitive sulfhydryl groups in capillary endothelium which might be involved in such reactions with arsenicals, and suggest, in conjunction with the work of Danielli, that the role of mercaptan groups of endothelium in regulating capillary permeability should be investigated further.

SUMMARY

A new red optical tracer compound, 1-(4-chloromercuriphenylazo)-naphthol-2, is introduced as a cytochemical reagent for the demonstration of sulfhydryl groups in tissue components of microscopic size. Evidence is presented that the binding of this optical tracer to tissues depends on the chloromercuri group on the tracer molecule. and on the presence of intact sulfhydryl groups on the tissue component. It is demonstrated further that the red sulfhydryl reagent can

---

[6] Note added in proof: Wald (Science, *113:* 287, 1951) and Wald and Brown (Fed. Proc., *10:* no. 1, 266, 1951) have recently reported that —SH groups are present and physiologically important in the rhodopsin molecule, which, in turn, is a substantial constituent of retinal rods.

be caused to bind to tissue by specific mercaptide linkages, and that binding by non-specific hydrogen bonds, salt linkages, or van der Waals forces does not enter into the reaction under the conditions cited.

By use of this method sulfhydryl has been demonstrated as a component of capillary endothelium, the trachea of insects, of retinal rods, and the axoplasm and cell bodies of certain peripheral nerves and ganglion cells of vertebrates and invertebrates.

LITERATURE CITED

ANSON, M. L. 1939 The reactions of denatured egg albumin with ferricyanide. J. Gen. Physiol., *23:* 247–261.

——— 1940 The sulfhydryl groups of egg albumin. J. Biol. Chem., *135:* 797–798.

——— 1941 The sulfhydryl groups of egg albumin. J. Gen. Physiol., *24:* 399–421.

ARNOLD, VINZENZ 1911 Über den Cysteingehalt der tierischen Organe. Ztschr. f. physiol. Chem., *70:* 314–325.

BENNETT, H. STANLEY, AND DAVID A. YPHANTIS 1948 1-(4-chloromercuriphenylazo)-naphthol-2. J. A. C. S., *70:* 3522.

BUFFA, EDMOND 1904 Sur une combinaison sulfurée des tissus animaux. J. de Physiol. et de Path. Gen., *6:* 645–654.

CHÈVREMONT, M., AND J. FRÉDERIC 1943 Une nouvelle méthode histochimique de mise en évidence des substances à fonction sulfhydrile. Application à l'épiderme, au poil et à la levure. Arch. de Biol., *54:* 589–605.

DANIELLI, J. F., MARY DANIELLI AND P. D. MITCHELL 1947 BAL-INTRAV: A new non-toxic thiol for intravenous injection in arsenical poisoning. Biochem. J., *41:* 325–333.

ELKINS, MARJORIE AND LOUIS HUNTER 1935 The azo-group as a chelating group. Part I. Metallic derivatives of o-hydroxyazo-compounds. J. Chem. Soc., pp. 1598–1600.

GIROUD, A. 1928 Protoplasma et glutathion. Compt. rend. Soc. de Biol., *98:* 376–377.

——— 1929 Recherches sur la nature chimique du chondriome. Protoplasma, *7:* 72–98.

——— 1930 Les substances à fonction sulfhydrile du protoplasma. Association des anatomistes. Comp. rend., 25th Rèunion and 3rd Congrès Federatif International, pp. 144–146.

——— 1931 Les substances à fonction sulfhydrile du protoplasma. Protoplasma, *12:* 23–41.

GIROUD, A., AND H. BULLIARD 1928 Glutathion et kératine. Association des anatomistes. Comp. rend., 23rd Rèunion, pp. 167–168.

Giroud, A., and H. Bulliard 1929 Substances à fonction sulfhydrile de l'épiderme. Association des anatomistes. Comp. rend., 24th Rèunion, pp. 248–250.

———— 1930 La kératinisation de l'épiderme et des phanères. Genèse des substances soufrées de la kératine. Arch. de Morphol. Gen. et Expér., vol. 29, 83 pages, Paris, Gaston Doin and Cie.

———— 1933 Réaction des substances à fonction sulfhydryle. Protoplasma, *19:* 381–384.

Gola, G. 1902 Lo zolfo e i suoi composti nell'economia delle piante. Malphigia, *16:* 368–392.

Hammett, Frederick S. 1933a The proliferative response of neurones to sulfhydryl and sulfoxide in the regenerating ganglion of *Nereis pelagica.* Protoplasma, *19:* 117–124.

———— 1933b The influence of sulfhydryl and its sub-oxidized derivatives on the developmental cycle of hydranths of the genus *Obelia.* Protoplasma, *19:* 510–540.

Heffter, A. 1908 Ueber die reduzierenden Bestandteile der Zellen. Med. Naturw. Arch., *1:* 81–103.

Hellerman, Leslie, Marie E. Perkins and W. Mansfield Clark 1933 Urease activity as influenced by oxidation and reduction. Proc. Nat. Acad. Sci., *19:* 855–860.

Herrmann, Heinz and Sylvia G. Moses 1945 Content and state of glutathione in the tissues of the eye. J. Biol. Chem., *158:* 33–45.

Hopkins, Frederick Gowland 1921 On an autoxidisable constituent of the cell. Biochem. J., *15:* 286–305.

Hughes, Walter L. 1950 Protein mercaptides. Cold Spring Harbor Symposia on Quantitative Biology, *14:* 79–84.

Joyet-Lavergne, Ph. 1927a Sur le rôle du chondriome dans le métabolisme cellulaire. Compt. rend. Soc. de Biol., *97:* 327–330.

———— 1927b Sur les rapports entre le glutathion et le potentiel d'oxydoréduction intra-cellulaire. Compt. rend. Soc. de Biol., *97:* 140–142.

———— 1928 Sur les rapports entre le nucléole, le chondriome et le glutathion. Compt. rend. Soc. de Biol., *98:* 567–569.

Junqueira, L. C., A. Fajer, M. Rabinovitch and L. Frankenthal 1949 Biochemical and histochemical observations on the sexual dimorphism of mice submaxillary glands. J. Cell. and Comp. Physiol., *34:* 129–158.

Kodomari, Shigehiko 1940 Über die Glutathion in der Netzhaut von Kaninchenaugen. I. Mitteilung: Über den Gehalt von Glutathion in der Netzhaut bei normalen Kaninchen und einige daran anschlessenden Experimente. Jap. J. M. Sc., X. Ophthalm., *2:* 574.

Mason, Frederick Alfred 1932 Structure of para red and related dyestuffs. J. Soc. Dyers and Colourists, *48:* 293–295.

Olcott, Harold S., and Heinz Fraenkel-Conrat 1947 Specific group reagents for proteins. Chemical Reviews, *41:* 151–197.

Pauling, Linus 1945 The nature of the chemical bond. 2nd Ed., Cornell University Press, Ithaca, New York.

ROSNER, LAWRENCE, C. J. FARMER AND JOHN G. BELLOWS 1938 Biochemistry of the lens. XII. Studies on glutathione in the crystalline lens. Arch. Ophthalm., *20:* 417–426.

SINGER, T. P., AND E. S. G. BARRON 1945 Studies on biological oxidation. XX. Sulfhydryl enzymes in fat and protein metabolism. J. Biol. Chem., *157:* 241–253.

SÜLLMAN, H., AND A. E. SCHMID 1942 Ueber die Oxydoreduktionssysteme der Netzhaut. I. Ascorbinsäure and Glutathion. Ophthalmologica, *103:* 150–155.

WALKER, ERNEST 1925 The sulphydryl reaction of skin. Biochem. J., *19:* 1085–1087.

PLATE 1

EXPLANATION OF FIGURES

All illustrations are of teased pieces of frozen alcohol-dried tissues of frog after immersion in 1-(4-chloromercuriphenylazo)-naphthol-2. The presence of red, pink, or orange color indicates the sites of binding of the red sulfhydryl reagent by tissue mercaptan groups.

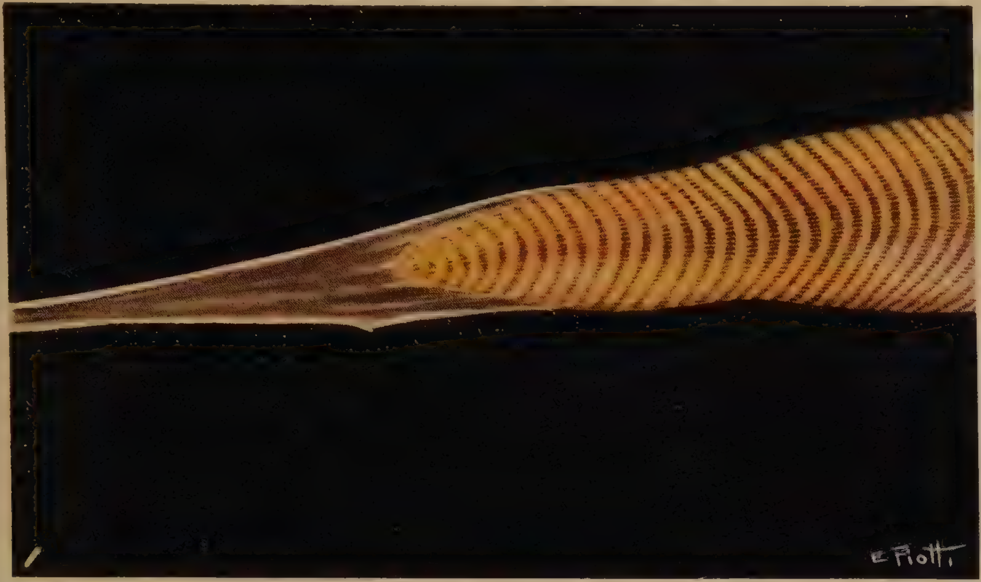
1 Skeletal muscle fiber of frog at myo-tendinal junction, shown as it appears between crossed polarizers. The red color in the contractile portion is in sharp contrast to the white of the tendon, indicating that the sulfhydryl groups are confined to the muscle proper. The prominent cross banding in the drawing is due primarily to polarization optical effects, and should not be construed as representing a variation in the disposition of sulfhydryl in accordance with the A and I bands.

2 A retinal rod teased from a frog's eye. A few pigment granules are adherent. The pink color is due to bound red sulfhydryl reagent, evidencing the presence of sulfhydryl in the rod.

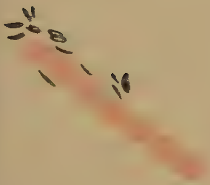
3 Teased sciatic nerve of frog. The pale orange color in the axoplam betokens the presence of sulfhydryl in this structure. The artist has portrayed the myelin sheaths as presenting slightly more contrast with the axoplasm than is actually the case. One cannot be certain from examining the original specimen that no sulfhydryl reagent is bound by the sheath.

4 Teased fragment from the cortical portion of the crystalline lens of the eye. Note the distribution of red, indicating a concentration of sulfhydryl along the surfaces of the lens fibers and in fine strands making a delicate tracery amongst them.

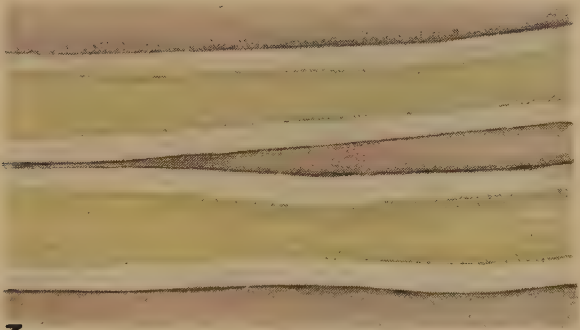
5 A capillary teased out from amongst frog skeletal muscle fibers. A few distorted erythrocytes are imprisoned in the dilatation, and show uptake of red sulfhydryl reagent, as does the endothelium itself.



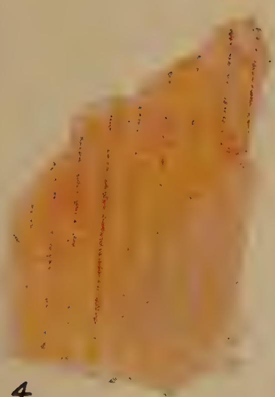
1



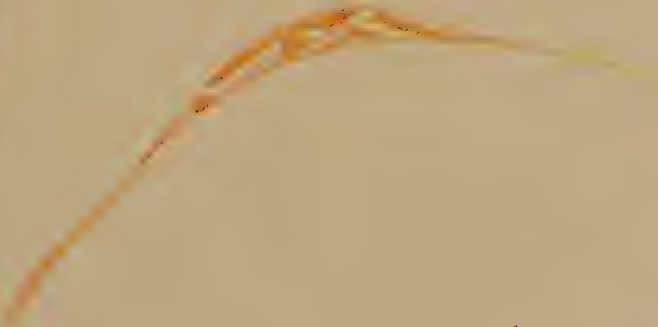
2



3



4



5

STUDIES ON THE HEART OF VITAMIN E DEFICIENT RABBITS[1]

ARTHUR J. GATZ AND O. BOYD HOUCHIN
Departments of Anatomy and Biochemistry, Stritch School of Medicine, Loyola University, Chicago

THIRTEEN FIGURES

The paralysis which developed in suckling rats of vitamin E deficient mothers was first described by Evans and Burr ('28). Olcott ('38) demonstrated that the paralysis was due to muscle lesions similar to those which were described by Goettsch and Pappenheimer ('31) in young guinea pigs and rabbits. The same dystrophic condition has been produced in other animals[2] on vitamin E deficient diets. The histological modifications in the skeletal muscle described by the above workers are accompanied by alterations in the functional behavior and in the chemical composition of the muscle. Houchin and Mattill ('42), Kuntz and Pappenheimer ('43), and Pappenheimer ('43) determined that the rate of oxygen consumption is increased in the skeletal muscle from vitamin E deficient animals. None of the above workers have reported any histological evidence of lesions in the cardiac muscle. Houchin and Smith ('44), in their attempt to determine the immediate cause of death, observed evidence of myocardial failure in vitamin E deficient rabbits. They also reported an increased O_2 consumption in the cardiac muscle from E-deficient hamsters.

---

[1] This problem has been supported in part by a grant from the American Medical Association Council on Medical Research to Dr. O. B. Houchin.

[2] Goettsch and Pappenheimer ('34; ducklings); Ringsted ('35; older rats); Madsen, McCoy and Maynard ('35; sheep and goats); Houchin ('42; hamster); Pappenheimer ('42; mice).

Freire ('41) reported focal hyaline necrosis in the myocardium of vitamin E deficient rats. Freire and Magalhaes ('43) reported focal hyaline necrosis in the subpericardial fibers of the heart and a QRS complex of low voltage in electrocardiographic studies of vitamin E deficient guinea pigs. Mason and Emmel ('45) reported necrosis and fibrotic replacement in the rat myocardium which was apparent after more than a year on an E deficient diet. They observed brown pigment globules in the somewhat vacuolated sarcoplasm. The myofibrillae maintained their integrity until necrosis occurred. The most marked lesions were apparent in the peripheral portion of the ventricles, though lesions also occurred in the interventricular septum and in the atrial musculature. No significant alterations in the blood pressure were observed in the experimental rats. Gatz and Houchin ('46 and '47) reported electrocardiographic, oxygen consumption and histological studies on vitamin E deficient rabbit hearts. Gullickson and Calverley ('46) found a decrease in the potential of the QRS complex and also noticed an increase in the duration of the PR, QRS and QT intervals in electrocardiographic studies on vitamin E deficient cattle. Mason and Telford ('47) described the gross and histological evidence of dystrophy in the skeletal muscle of vitamin E deficient monkeys. The deposition of small amounts of pigment in the muscle fibers was observed. The cardiac muscle fibers contained some pigment but showed little or no evidence of damage.

MATERIAL AND METHODS

Forty-four (44) littermate New Zealand giant rabbits, 5 weeks of age and weighing about 600 grams, were separated into control and experimental groups. One half of the latter were placed on the following vitamin E deficient diet: Cellu flour 20%, casein (acid washed) 15%, starch (alcohol-ether extracted) 46%, lard 3%, cod liver oil 3%, salt mixture[3] 3%

---

[3] Hubbell, R. B., L. B. Mendel and A. J. Wakeman. J. Nutrition, 1937, *14:* 273.

and yeast (dried brewer's) 10%. The control animals in this series received the regular laboratory ration of alfalfa hay and grain, supplemented with lettuce, cabbage and carrots. The control animals did not show any of the signs of vitamin E deficiency.

In the second series of experiments, the E-deficient diet was varied as follows: lard 9%, crystalline carotene 5 mg/kilo and viosterol (Mead's) 0.2 mg/kilo (substituted for cod liver oil) and vitamin K 1.0 mg/kilo. The experimental groups were placed on this deficient diet and the control groups received the same ration supplemented with ephynal acetate (25 mg/kilo). All of the rabbits grew at the same rate through the third week. During the 4th week the E-deficient rabbits exhibited marked weight losses and the following signs of nutritional muscular dystrophy: a purulent secretion from the eyes, muscular weakness and paralysis. The experimental rabbits were used for study when they were unable to rise after being placed on their side. None of the controls receiving the ephynal acetate fortified ration showed any deficiency signs.

The electrocardiograms were taken with a General Electric, Victor, Model A electrocardiograph. The procedure which was used has been described by Houchin ('46). The experimental rabbits were untreated prior to the attachment of the leads. The control animals usually allowed the leads to be attached without anesthesia, though it was occasionally necessary to inject (10 ml/kilo) pentothal sodium. If the latter was necessary, the animals were allowed to regain consciousness before the record was taken.

The rabbits were sacrificed by means of an air embolism to remove the tissues used for oxygen consumption and histological studies. The O_2 consumption of the cardiac muscle slices was measured in oxygen at 38°C. by the usual Warburg procedure in modified Locke's solution. The procedure followed was described by Houchin and Mattill ('42). Other portions of the heart and skeletal msucle, used for histological study, were fixed in Bouin's fluid. The thyroids and adrenals were fixed in Zenker's-formol fluid. The Mallory connective tissue stain was found to be most useful for the study of the cardiac muscle and the thyroids.

RESULTS

Oxygen consumption. The results of the metabolism study on the cardiac muscle slices from the rabbit verified the previous findings of Houchin and Smith ('44). The results, as indicated in table 1, are expressed as Q_{O_2} (cubic millimeters of oxygen taken up per milligram of dry weight of tissue per hour).

TABLE 1

ANIMAL	Q_{O_2} FOR CARDIAC MUSCLE		INCREASE
	Control	Experimental	
no.	*mm³ O₂*	*mm³ O₂*	%
5	4.1	4.9	20
6	3.8	4.6	21
7	3.7	6.9	86

The correlation of the results of the metabolic studies of these animals with the histological findings is presented under the histological results.

Electrocardiographic results. The electrocardiograph was used to determine the condition of the heart before the animal was sacrificed. The records presented in figure 1 indicate the progressive changes that occurred in experimental rabbit 4. The heart rate was slightly above normal on August 11, 1945, which was followed by a gradual decrease to a rate below normal on August 14, 1945. The P and T waves show a gradual reduction in potential. The decrease in the amplitude of T_2 may indicate a functional impairment of the myocardium. The potential of the QRS_1 complex was reduced on August 14th and the QRS_3 changed from the RS type to the S type on August 13th which may indicate myocardial damage.

A right axis deviation, which is indicated in the records shown in figure 2, was observed in many of the E-deficient

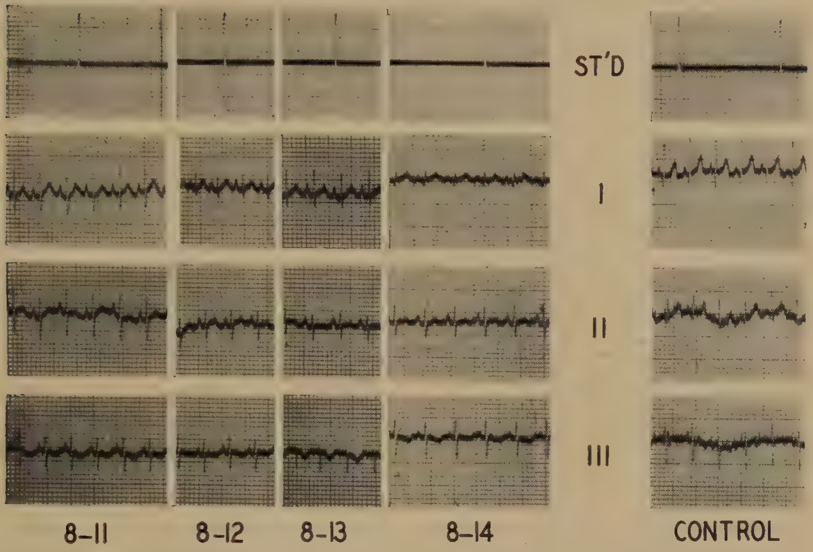


Fig. 1 Electrocardiograms of vitamin E deficient rabbit no. 4 on 4 successive days.

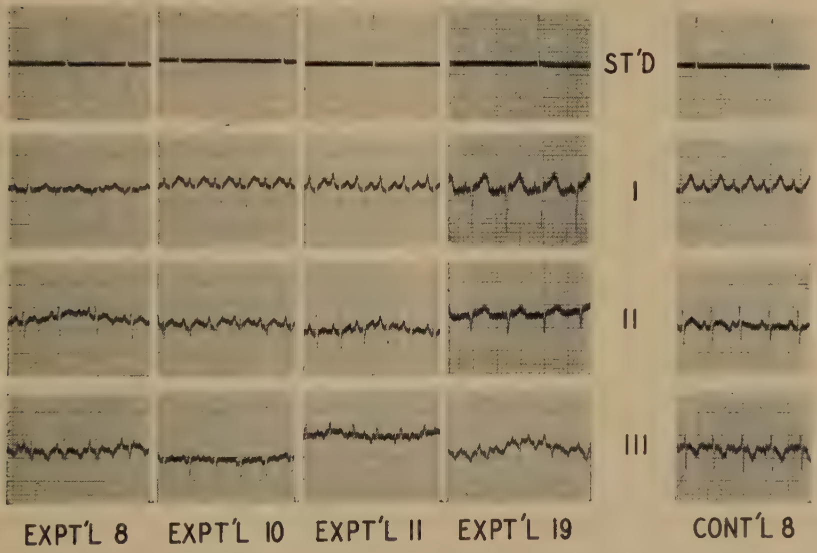


Fig. 2 Electrocardiograms of some vitamin E deficient rabbits showing right axis deviation.

rabbits. In right axis deviation the main deflection of QRS_1 is downward and R_3 is greater than R_2, both of which may be observed in these records. T_3 is inverted in practically all of the E-deficient records, though it is isoelectric in some. The T_1 and T_2 waves of the deficient rabbits show some notching. The notching of T_1 and T_2, the inverted potential of $QRS_{1 \text{ and } 3}$ and the decreased potential of all T waves are indications of myocardial damage.

No signficant variations in the PR and QRS intervals have been observed in the experimental animals. The QT interval was shortened in some instances. The correlation of the electrocardiographic results with the oxygen consumption records showed that the rabbits exhibiting shortened QT intervals also had a higher metabolic rate. These results have also been reported by Robb ('46). The electrocardiograms of all control animals, including those on the E-deficient ration supplemented with ephynal acetate, were normal in appearance.

Histological study. The hearts of the experimental rabbits appeared slightly larger than those of the control animals. The cardiac muscle failed to show any gross modifications. The skeletal muscles of the limbs of the E-deficient rabbits appeared moist and pale and responded with negligible contraction when cut as opposed to the vigorous contraction of the muscles of the control animals. There was no evidence of pigmentation in the cardiac and skeletal musculature.

The histological structure of the experimental rabbit cardiac muscle parallels the description for Zenker's hyaline degeneration and the necrosis in skeletal muscles described by Pappenheimer ('31, '39, '43) and Olcott ('38). The papillary muscles of the left ventricle with the associated ventricular wall, the left atrium and the interventricular septum were used for the histological observations. A considerable number of hyalinized fiber areas in the papillary muscle and the associated left ventricular wall of an E-deficient rabbit are shown in figure 3. The basophilic hyalinized and necrotic fibers of another E-deficient animal are shown under higher magnification in figure 4.

The appearance of the cardiac muscle fibers in the control rabbits is shown in figure 5. The striations are conspicuous in the fibers which are supported by a minimal amount of endomysial connective tissue containing the capillaries. In the cardiac tissue of E-deficient rabbit 7 (fig. 6), which showed the pronounced increase in O_2 consumption, the cardiac capillaries and other vessels are prominent and distended with blood. Other areas of the heart showed some evidence of basophilic hyalinized fibers. However, the amount of basophilia, the appearance of constriction bands and necrosis were not as prominent as those observed in the cardiac tissue of E-deficient rabbits 5 and 6 (fig. 7). These results seem to indicate that the muscle metabolism becomes increased previous to the appearance of necrotic lesions in the tissue. Hence, the rate of oxygen consumption of the E-deficient rabbit's cardiac muscle returns to the normal level as the necrotic areas become more numerous and the normal cardiac fibers become reduced in amount as a consequence.

Throughout the entire series of E-deficient rabbits, small vacuoles occurred in the sarcoplasm of the ventricular myocardium (fig. 8) or the papillary muscle. At the present time, the contents of the vacuoles are unidentified. The vacuoles do not contain fat, since they are unstained with osmic acid, or an acid fast pigment because the droplets fail to react with the Ziehl-Nielson stain. The vacuoles occurred in the scattered hyalinized basophilic fibers (fig. 8) or in the normally stained cardiac fibers. The myofibrillae become more widely separated as the droplets enlarge causing the Q bands to assume a granular appearance. As the droplets coalesce, there is a gradual loss of myofibrillae (fig. 9) until the cell eventually consists of the sarcolemma containing the coalesced fluid in which a much constricted nucleus may be apparent. Mitotic figures were not observed in the cardiac fibers, though the nuclei frequently showed constrictions and appeared to be dividing amitotically. Necrotic areas were infrequently observed in the cardiac fibers of the left atrium. The Purkinje fibers apparently were not involved in the degenerative process. The necrosis which occurred in the rabbits on E-deficient diets without cod liver oil (fig. 3) was more severe than that occurring in the E-deficient rabbits on the diet which contained cod liver oil. The control rabbits in both series failed to show any evidence of necrotic lesions. An interstitial edema (partially fibrous) accompanied the alteration of the cardiac fibers and caused the separation of the cardiac cells (fig. 10). Polymorphonuclear cells were quite numerous in the early stages of the affected endomysial regions of the heart. The connective tissue later became infiltrated with mononuclear histiocytes.

The observations on the tibialis anterior muscle of the E-deficient rabbits (fig. 11) were similar to those described for the myocardium, in our study, and to those described previously by Pappenheimer ('31, '39, '43) and Olcott ('38).

The histological study of the thyroids was undertaken in order to ascertain if they were involved in the increased O_2 consumption rate of the cardiac muscle. The glands of the control animals showed an active epithelium and the follicles contained active colloid. The thyroid glands of the E-deficient rabbits were inactive as determined by the presence of the stored colloid and the flattened follicular epithelium.

The cardiac plexus, which is located between the bases of the pulmonary artery and the aorta, was studied during the observations on the interventricular septum. The neurons (figs. 12 and 13) and the nerve fibers of the plexus were normal in appearance. Previous studies on the motor end plates of skeletal muscle have shown that the nerve endings are unaffected by nutritional muscular dystrophy in guinea pigs (Rogers, Pappenheimer and Goettsch, '31). Pappenheimer ('39) reported that, "even terminal neurites and end-plates were well preserved in the midst of degenerated muscle fibers," in the E-deficient rat skeletal muscle. Telford ('41) studied the E-deficient rat skeletal muscle and found that there was no appreciable loss of nerve endings if the muscle degeneration was slight. A necrosis which was severe and of long standing caused a complete loss of motor end plates. All of the above reports maintained that vitamin E deficiency has no direct effect on the nerve endings though Telford stated that, "the extent, degree and duration of the degenerative process is important in interpreting these changes." Our results on the cardiac plexus verify the previous findings on skeletal muscle. In view of the physiological necessity for the heart, it is inconceivable that cardiac muscle degeneration could ever be of sufficient duration to allow observations of nerve degeneration.

DISCUSSION

The oxygen consumption findings and the results of the histological studies on the cardiac muscle of vitamin E deficient rabbits show a significant correlation. The experimental animals which showed an increased muscle metabolism revealed the early phases in the necrosis of the cardiac muscle. We believe that an initial change in E-deficient cardiac fibers is the increased muscle metabolism which causes the histological modifications (hyalinization, constriction bands and basophilia) to appear. As the necrotic changes become more severe, the oxygen consumption, though above normal, is not as great as in the early stages. The thyroid apparently has no active role in the deficiency syndrome. In our experiments the thyroids of the vitamin E deficient rabbits contained stored colloid and the follicular epithelium was inactive. The experimental work of Houchin and Mattill ('42), who showed that the addition of alpha tocopherol phosphate dissolved in Ringer-Locke solution brings about a marked reduction in the oxygen consumption of vitamin E deficient skeletal muscle, is also evidence against the participation of the thyroid in these results. Vitamin E apparently is an essential participant in the enzyme system of the muscle cell and exerts a controlling influence on the metabolism of the muscle.

There also is a correlation between the electrocardiographic and histological studies. The necrosis was more severe in the experimental rabbits which showed right axis deviation than it was in those which failed to show this condition. The Purkinje fibers were not affected histologically which coincides with the electrocardiographic evidence of normal QRS intervals in the experimental rabbits.

The gross signs of nutritional dystrophy and cardiac necrosis occurred more rapidly following the consumption of the experimental diet in which vitamin D was supplied as irradiated ergosterol than it did from the diets which contained cod liver oil. It is also of interest to find that the cardiac plexuses were maintained in excellent condition even though considerable necrotic tissue was apparent in the vitamin E deficient hearts.

SUMMARY

Myocardial damage of sufficient magnitude to cause the death of vitamin E deficient rabbits has been observed. We believe that the physiological and morphological changes occurred in the following sequence:

1. An increase in the muscle metabolism which was shown by the increase in oxygen consumption and by the lengthened QT interval in the E-deficient cardiac muscle electrocardiograms.
2. The affected cardiac fibers undergo marked contraction as determined by the appearance of constriction bands. The affected fibers became hyaline in appearance and exhibited basophilia.
3. Fluid droplets, the composition of which are unknown as yet, appeared in the sarcoplasm between the myofibrils. As the droplets coalesced, the Q bands were separated and presented a granular appearance. Eventually the affected fibers consisted of the sarcolemma which surrounded the coalesced droplets and the muscle nuclei.
4. An interstitial edema caused the separation of the cardiac fibers. The polymorphonuclear cells and histiocytes invaded the necrotic area.
5. The portions of the heart showing necrosis of cardiac muscle may be listed as follows with regard to the frequency

and severity of the lesion: papillary muscle, ventricular walls and septum, and infrequently the atrial walls. The Purkinje fibers appear unaffected.

6. The neurons and the nerve fibers of the cardiac plexus appeared to be unaffected by the deficient diet.

7. The variations produced in the electrocardiographic records are indicative of myocardial damage.

LITERATURE CITED

EVANS, H. M., AND G. O. BURR 1928 Development of paralysis in the suckling young of mothers deprived of vitamin E. J. Biol. Chem., *76:* 273–297.

FREIRE, S. A. 1941 Necrose hyalina focal do miocárdio do rato par hypovitaminose E. Brasil Med., *55:* 308–310.

FREIRE, S. A., AND B. F. MAGAHAES 1943 O miocárdio na avitaminose e da Cobáia. Rev. Brasil de Biol., *3:* 91–98.

GATZ, A. J., AND O. B. HOUCHIN 1946 The histology of vitamin E deficient rabbit hearts. Anat. Rec., *94:* 462.

———— 1947 Histological observations on the vitamin E deficient rabbit heart. Anat. Rec., *97:* 337.

GOETTSCH, M., AND A. M. PAPPENHEIMER 1931 Nutritional muscular dystrophy in the guinea pig and rabbit. J. Exp. Med., *54:* 145–165.

———— 1934 Nutritional myopathy in ducklings. J. Exp. Med., *59:* 35–42.

GULLICKSON, T. W., AND C. E. CALVERLEY 1946 Cardiac failure in cattle on vitamin E-free rations as revealed by electrocardiograms. Science, *104:* 312–313.

HOUCHIN, O. B. 1942 Vitamin E and muscle degeneration in the hamster. Federation Proc., *1:* 117–118.

———— 1946 A simple electrocardiograph contact electrode for use with the smaller laboratory animal. J. Lab. and Clin. Med., *31:* 595–597.

HOUCHIN, O. B., AND H. A. MATTILL 1942 The oxygen consumption, creatine and chloride content of muscles from vitamin E-deficient animals as influenced by feeding tocopherol. J. Biol. Chem., *146:* 301–307.

———— 1942 The in vitro effect of tocopherol and its phosphate derivative on oxidation in muscle tissue. J. Biol. Chem., *146:* 313–321.

HOUCHIN, O. B., AND P. W. SMITH 1944 Cardiac insufficiency in the vitamin E-deficient rabbit. Am. J. Physiol., *141:* 242–248.

KAUNITZ, H., AND A. M. PAPPENHEIMER 1943 Oxygen consumption in vitamin E-deficiency. Am. J. Physiol., *138:* 328–340.

MADSEN, L. L., C. M. MCCOY AND L. A. MAYNARD 1935 Synthetic diets for Herbivora with special reference to the toxicity of cod liver oil. Cornell Univ. Agric. Exp. Station Memoir, *178:* 3–53.

MASON, K. E., AND A. F. EMMEL 1945 Vitamin E and muscle pigment in the rat. Anat. Rec., *92:* 33–59.

MASON, K. E., AND I. R. TELFORD 1947 Some manifestations of vitamin E-deficiency in the monkey. Arch. Path., *43:* 363–373.

OLCOTT, H. S. 1938 The paralysis in the young of vitamin E deficient female rats. J. Nutrition, *15:* 221–228.

PAPPENHEIMER, A. M. 1939 The pathology of nutritional muscular dystrophy in young rats. Am. J. Path., *15:* 179–184.

——— 1942 Muscular dystrophy in mice on vitamin E deficient diet. Am. J. Path., *18:* 169–182.

——— 1943 Muscular disorders associated with deficiency of vitamin E. Physiol. Rev., *23:* 37–50.

RINGSTED, A. 1935 A preliminary note on the appearance of paresis in adult rats suffering from chronic avitaminosis E. Biochem. J., *37:* 788–795.

ROBB, J. S. 1946 A study of QT interval in various species. Federation Proc., *5:* 87.

ROGERS, W. M., A. M. PAPPENHEIMER AND M. GOETTSCH 1931 Nerve endings in nutritional muscular dystrophy in guinea pigs. J. Exp. Med., *54:* 167–169.

TELFORD, I. R. 1941 Loss of nerve endings in degenerated skeletal muscles of young vitamin E deficient rats. Anat. Rec., *81:* 171–181.

PLATES

PLATE 1

EXPLANATION OF FIGURES

ABBREVIATIONS

BD, basophilic degeneration	MN, muscle nucleus
C, cytolysis	N, nerve
CB, constriction band	RC, round cell
CC, cardiac capillary	V, vacuole
GC, ganglion cells	

Bouin's fixation and Mallory's connective tissue stain were used in all sections except figure 4.

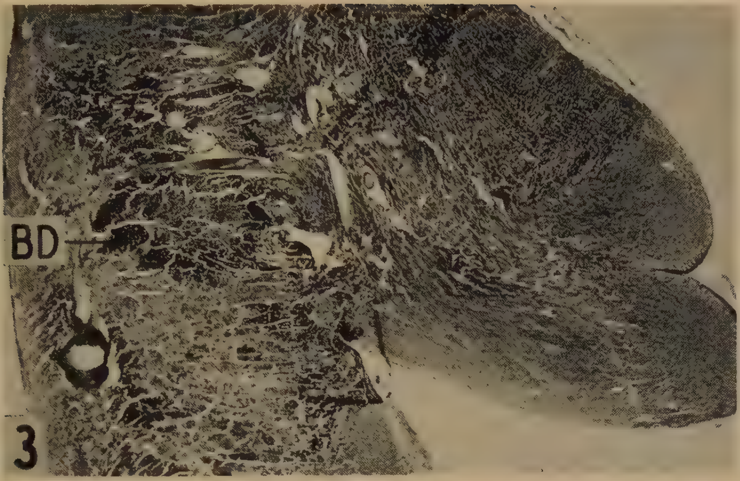
3 Papillary muscle and portion of left ventricle from E-deficient rabbit 15. Numerous areas showing basophilic degeneration. × 14.

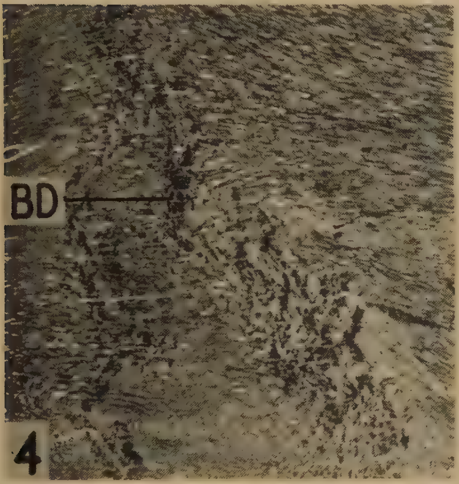
4 Longitudinal section of papillary muscle from E-deficient rabbit 19. Basophilic degeneration extends across the papillary muscle. Cytolysis of some fibers is apparent. Ludford's fixation. × 20.

5 Longitudinal section of papillary muscle from control rabbit 5. Nuclei and striations are conspicuous. × 550.

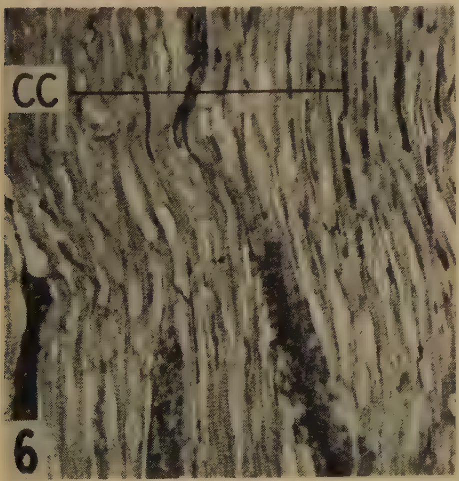
6 Longitudinal section of papillary muscle from E-deficient rabbit 7, which is the animal that exhibited an 86% increase in oxygen consumption. Notice distension of cardiac vessels with blood. × 280.

7 Longitudinal section of papillary muscle from E-deficient rabbit 5. Nutritional muscular dystrophy is markedly advanced. Section shows basophilic areas, constriction bands, cytolysis of fibers with loss of myofibrillae and vacuoles in the sarcoplasm. × 550.


BD
3


BD
4

5


CC
6

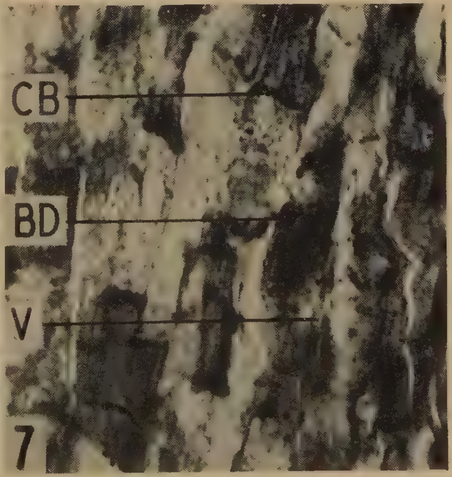

CB
BD
V
7

PLATE 2

EXPLANATION OF FIGURES

8 Transverse section of left ventricle of E-deficient rabbit 8. Numerous fibers show basophilic degeneration. Vacuoles of unidentified material are present in the hyaline-like and normal fibers. × 550.

9 Longitudinal section of papillary muscle from E-deficient rabbit 8. Nutritional muscular dystrophy is considerably advanced. Vacuoles of unidentified material have coalesced. × 550.

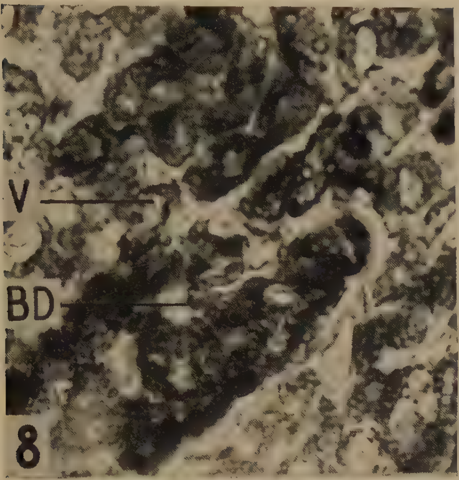
10 Longitudinal section of papillary muscle from E-deficient rabbit 4. Muscle has almost lost identity. Some muscle nuclei are apparent and round cells have infiltrated area. × 550.

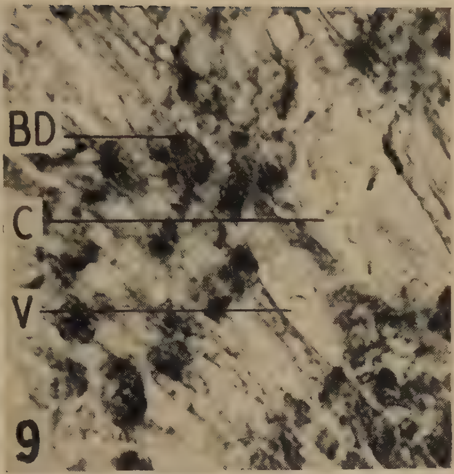
11 Longitudinal section of tibialis anterior muscle from E-deficient rabbit 8. Nutritional muscular dystrophy resembles that observed in the cardiac muscle. × 550.

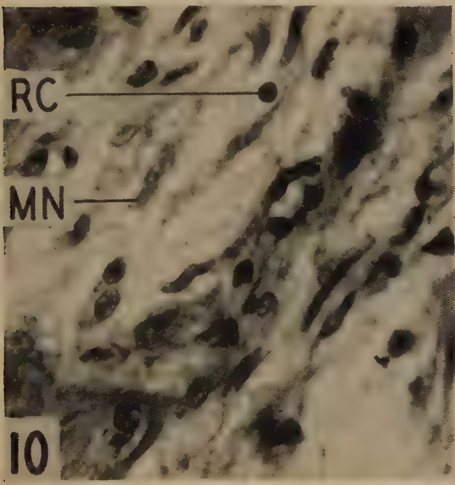
12 Section of cardiac plexus from control rabbit 19. Ganglion cells are surrounded by capsule cells and sections of two small nerves of plexus. × 300.

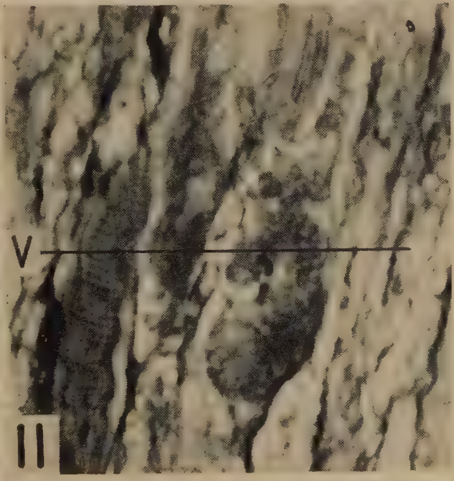
13 Section of cardiac plexus from E-deficient rabbit 19. Two groups of ganglion cells and a section of a nerve are shown. × 175.

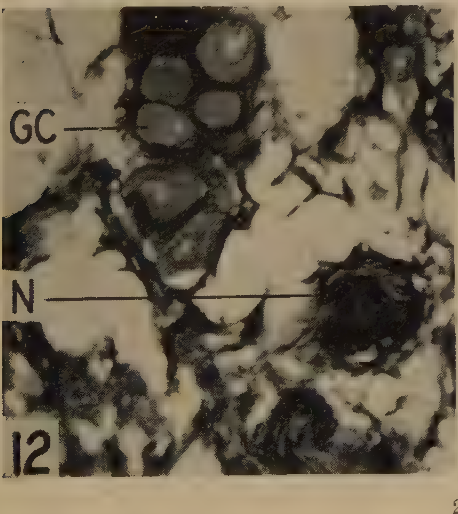
BD, Basophilic degeneration
C, Cytolysis
CB, Constriction band
CC, Cardiac capillary
GC. Ganglion cells
MN, Muscle nucleus
N, Nerve
RC, Round Cell
V, Vacuole

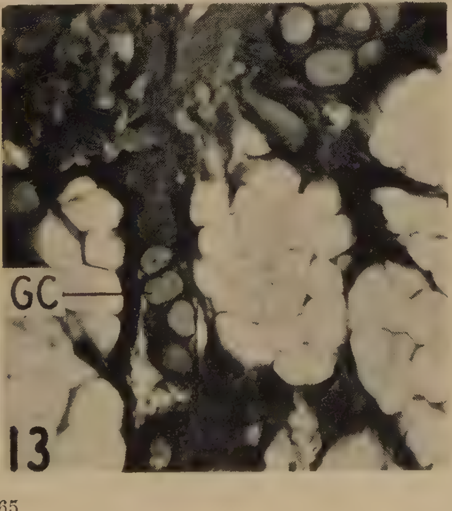

V
BD
8


BD
C
V
9


RC
MN
10


V
11


GC
N
12


GC
13

THE REACTION OF THE LUNG IN THE CAT TOWARD OIL DROPLETS IN THE CIRCULATION

R. M. WOTTON AND A. MARTIN, JR.
The Department of Biological Sciences, The University of Pittsburgh, Pittsburgh, Pa.

FIVE FIGURES

INTRODUCTION

Wotton and Zwemer ('39), while studying the absorption of previously stained fat in the cat, noted that this colored lipid could be traced by way of the blood circulation to, and throughout the lung. These investigations suggested that some of the fat was excreted into the alveolar spaces of the lung. This is noteworthy, because only waste materials in a gaseous state are believed to be lost through the normal lung; and it seemed desirable to test by further work on the cat lung the correctness of our earlier observations.

PROCEDURE

A series of 18 kittens were fed test doses of 5–10 cm^3 of cod liver oil saturated with Sudan IV by passing the tip of a small rubber tube (catheter no. 22) gently through the mouth and down the esophagus into the stomach. Even pressure on the plunger of a glass syringe containing a measured amount of oil, and attached to the free end of this tube, delivered the test meal into the animal.

After one hour each animal was sacrificed by a stunning blow on the head. This was done to avoid the action of anaesthetic agents on the respiratory epithelium. The trachea first was dissected out in the neck region and clamped off to prevent the collapse of the lungs following penumothorax.

Then the chest cavity was opened, and the lungs removed and fixed in 10% pyridine formalin (Burke, '36).

After 24 hours of fixation, sectors of tissue were cut from the whole lung in those regions which showed pink areas indicating the presence of stained fat. These pieces of tissue were washed in running water overnight, infiltrated in an incubator in 1%, 5% and 10% gelatin solutions respectively, and imbedded in petri dishes in 10% gelatin. When this had cooled, the dishes were placed in the ice box until the gelatin mass had solidified, at which time blocks containing the lung tissues were cut out and immersed in 10% pyridine formalin to complete their hardening and to serve as a preservative for storage.

After washing the blocks in running water overnight sections were cut at 8–10 μ on a sliding microtome equipped with a freezing attachment, spread in a dish of distilled water, and transferred to slides. A drop of 1% gelatin was run under each section to act as a binding agent to secure it to the slide during subsequent technical treatment (Zwemer, '33). Slides were allowed to remain on the warming table until the sections appeared to be almost dry before they were immersed in 10% formalin.

All sections were washed in running water before staining. Some were dipped in Herxheimer's fat stain to intensify the color already present in the ingested oil, and counterstained in phosphotungstic acid hematoxylin, or Heidenhain's iron alum hematoxylin for nuclear and other cytoplasmic details. Others were merely stained in hematoxylin according to Harris' formula to serve as controls. All sections were mounted permanently under cover glasses in glychrogel (Zwemer, '33; Wotton and Zwemer, '35; and Wotton, '37).

OBSERVATIONS

Red colored fat was present in the lung within the phagocytic cells, the blood vessels, and free in the interstitial and alveolar spaces. The "dust cells" located in the walls of the alveoli and their septa were heavily loaded with stained fat (figs. 1–5). In the alveolar ducts in certain regions these cells formed a single row lining the cavity (fig. 1). Other cells similar in appearance to histiocytes also were loaded with oil droplets. In many instances they contained black particles as well as fat, and were present in the alveoli and interstitial spaces of the lung parenchyma.

The droplets of lipid were demonstrated within the lumina of small blood vessels at their sides, closely adjacent to the intima of the vessel (fig. 1), and in many instances were held between the endothelial cells of the arterioles and capillaries.

Finger-like projections of fat extending outward from the edge of the respiratory epithelium and large spherical droplets free within the spaces of the alveoli were observed. The presence of lipid in lung tissues was especially prominent in the subplural regions.

DISCUSSION

The lung receives a double blood supply; the main circulatory channel, however, is by way of the pulmonary arteries and veins. A major portion of the lipid absorbed from the small intestine and carried by the thoracic duct would reach the lungs first, before passing out through the systemic circulation. Fat carried by the mesenteric veins, on the contrary, before making its way to the pulmonary circulation would have to pass through the hepatic portal system. During such transit the liver would remove almost all of the lipid material (Wotton and Zwemer, '39, their figs. 7–11). It would seem reasonable to conclude that most of the oil came through the pulmonary arteries, and that little if any was carried to the lungs by way of the bronchial arteries.

Oil droplets and globules make their way through the pulmonary arteries down into the arterioles and capillaries surrounding the alveoli of the subpleural zones of the lung. Here they are squeezed out between the endothelial cells lining these blood vessels; and then pushed through the surrounding tissue spaces. From here, unless they be phagocyted first by the dust cells and histiocytes, they are extruded into the alveolar spaces. This passive diapedesis and movement of oil droplets may be accounted for largely on the basis of hydrostatic forces generated by variations in blood pressure combined with the fluctuations of intrathoracic pressures. Such differences in negative pressures, below that of atmospheric, found within the thorax exert a suction or pull upon the blood column.

Pulmonary arterial pressure falls during inspiration; but at this time, because of the traction exerted upon the blood vessels by the surrounding elastic lung tissue their capacity is actually increased. During expiration the forces are reversed, so that as the blood pressure rises, the walls of the arteries are contracting (Best and Taylor, '50). It can be seen that the application of these combined forces would account readily for the transit of a highly plastic material like oil through a very small aperture between the endothelial cells. At the same time other structures, less pliable, such as blood cells would be retained. Wotton and Zwemer ('39) have reported on this differential diapedesis of fats between the endothelial cells of the lacteals in the villi of the small intestine. Here also negative pressure was a factor. Further, it was noted that the excretion of oil was greatest in the subpleural zones, or in those regions of maximum distensibility of the lung tissue.

Foreign materials normally are removed from the air passages and spaces of the lungs by coughing, the action of ciliated cells, the peristaltic movements of the bronchioles and by phagocytosis. The possibility that any fat could have entered the alveoli by way of the trachea may be excluded when we consider that it would have had to make its way against such obstacles before arriving in the subpleural regions. No fat was found in the trachea, nor did the animals cough up any oil after being fed. Further, the presence of oil droplets in the blood vessels, and their diapedesis into the adjacent lung tissue would clearly indicate that the blood stream was responsible for their transportation to the lung;

and, unlike particulate matter which on injection into the bronchial or pulmonary vessels passes untouched through the lung, they were extruded into the alveolar spaces. Here their presence, like air borne foreign particles, stimulated the phagocytic activity of the dust or septal cells which could be seen in all stages of ingestion of red oil and desquamation from the aveolar walls.

SUMMARY

Following a hematogenous dissemination, the extrusion of previously stained cod liver oil in normal cat lungs has been described in a series of 18 animals.

This diapedesis of oil droplets has been accounted for on the basis of forces generated by fluctuations in blood pressure and interthoracic pressures during respiration.

Histiocytes in the interstitial spaces, and dust or septal cells located in the alveolar walls were active in the phagocytosis of this foreign lipid material.

LITERATURE CITED

Best, C. H., and N. B. Taylor 1950 The Physiological Basis of Medical Practice. Williams and Wilkins Co., Baltimore. 333–334 and 354–355.

Burke, F. V. 1933 The pH of formalin. A factor in fixation. Am. J. Path., *9:* 915–920.

Wotton, R. M. 1937 The application of glychrogel mounting for trematodes. Stain Tech., *12:* 145–146.

Wotton, R. M., and R. L. Zwemer 1935 A note on "Glychrogel" mounting solution. Stain Tech., *10:* 21–22.

——— 1939 Studies on direct and visible ingestion of fat by differentiated body cells of the cat. Anat. Rec., *75:* 493–507.

Zwemer, R. L. 1933 A method for studying adrenal and other lipoids by a modified gelatin embedding and mounting technique. Anat. Rec., *57:* 41–44.

PLATE 1

EXPLANATION OF FIGURES

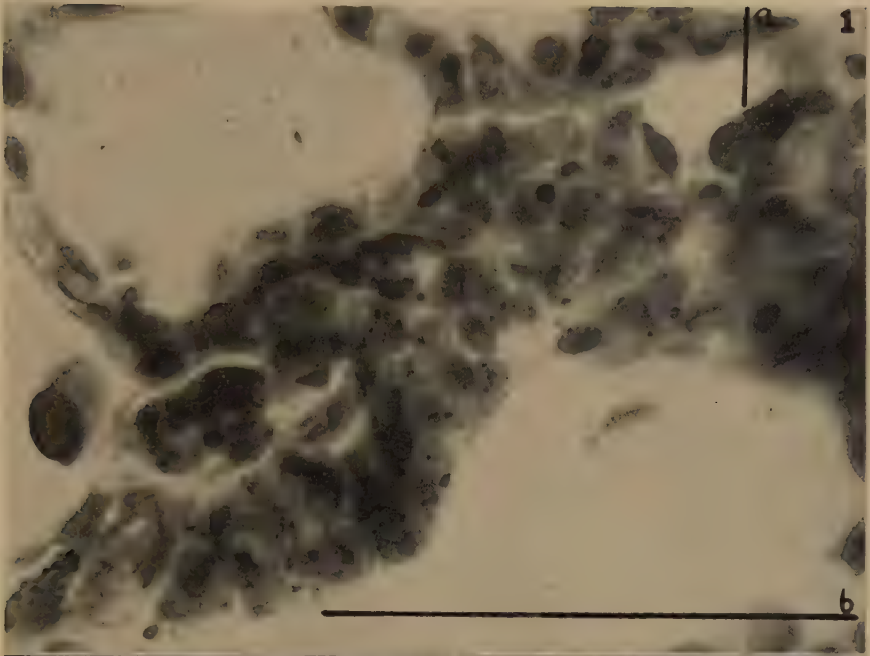
1 Section through an alveolar duct and several adjacent alveoli. a, Oil drops in a capillary. b, Epithelial cells of the alveolar duct containing numerous oil drops. × 450.

2 A septal cell ingesting several oil drops. × 1200.

3 A septal cell which has desquamated from the alveolar wall. × 1200.

4 Diagram of cell in figure 2.

5 Diagram of cell in figure 3.



4

5

THE FILIFORM AND THE CONICAL PAPILLAE OF THE TONGUE IN THE WHITE RAT[1]

HELEN KUTUZOV AND HARRY SICHER
Department of Histology, University of Illinois College of Dentistry, and Department of Anatomy and Histology, Loyola University School of Dentistry, Chicago

SIX FIGURES

INTRODUCTION

During the investigation of the effects of roentgen irradiation upon the tongue of the rat, it was found necessary to examine more minutely the normal gross and histological anatomy of this organ. While Fish, Malone, and Richter ('44) gave a satisfactory description of the fungiform, vallate, and foliate papillae, their description of the filliform and conical papillae is not adequate. The attempt, therefore, will be made to give a complete and detailed description of the filiform and the conical papillae of the white rat, thus establishing the basis for evaluation of experimental changes.

REVIEW OF LITERATURE

Sonntag ('24) describes the tongue in the Muridae as long, narrow, and thick, tapering or spatulate. He describes in Mus musculus the presence of innumerable small conical papillae and the characteristic single median circumvallate papilla. It is of a considerable size, round or flattened, with its surface smooth or granulated by small secondary papillae. According to Sonntag the fungiform papillae in Muridae are scanty and in many species have a solitary taste bud. In reference to the conical papillae he says that "they are numerous, minute, and close together. Those on the base of the tongue may be larger than those on the oral part of the tongue. Their apices may be entire or bifid, and they are covered with strongly cornified epithelium."

---

[1] This investigation was supported in part by a cancer training grant from the National Cancer Institute of the National Institute of Health, U. S. Public Health Service.

Fish, Malone, and Richter ('44) describe in some detail the superficial anatomical characteristics of the rat tongue and the frequency and the distribution of its papillae. The rats used in their investigation were crosses of the albino Wistar stock and the hooded stock from the colony of E. V. McCollum. Some rats were albino, others black or tan hooded, others all tan. According to these authors the filiform papillae are distributed over the entire surface of the body of the tongue. They appear externally as cuticular spines curved posteriorly, the largest spines being located in a broad patch on the forward portion of the dorsal prominence and the shortest on the sides and the under part of the tip. Elsewhere on the surface of the tongue they maintain an intermediate and constant size. Most of their epithelial portions lie beneath the level of the outer surface of the tongue with only a small cone-shaped tip entering the base of the spine. The epithelium consists of many layers of squamous cells with the superficial layer showing a rapid transition into the cuticular material of the spines. Their dermal cores are conical, often pointed, and fairly uniform in height.

Ziskin, Stein, Gross, and Runne ('47) state that the dermal cores of the posterior filiform papillae of the rat tongue are cylindrical, those of the anterior portion conical, while those of the tip follow both patterns. They are covered by heavily cornified epithelium which fills the grooves between them. The cornified spines of the papillae are directed posteriorly and are more compact on the posterior portion of the tongue than on the anterior. The rete pegs are narrow and cylindrical in the posterior region, but are irregular in arrangement and shape toward the tip of the tongue.

MATERIAL AND METHODS

This study is based on the gross and histologic examination of 21 rat tongues. The animals were albino rats, males and females, ranging in age from 10 days to 5 months, raised and maintained on purina pellets. All tongues were dissected out after the entire head had been fixed in 10% formalin. After fixation all material except one specimen was embedded in celloidin. Twelve specimens were cut serially in sagittal, transverse, or horizontal planes and were stained with hematoxylin-eosin. Eight specimens were cut sagittally, and the central sections of each specimen were staind with hematoxylin-eosin, Mallory's triple connective tissue stain, and Gomori's silver impregnation method for reticular fibers. From one specimen frozen sections were stained with Carlier's (1893) hematoxylin-benzopurpurin stain for horn and eleidin granules, with Buzzi's (Cowdry, '48) picric acid-nigorsin stain for horn and eleidin, and Martinotti's ('24) crystal violet-eosin B stain for eleidin, pre- and post-eleidin.

Sections of skin removed from the dorsal and ventral surfaces of a human toe were stained with all of the above mentioned stains for the purpose of comparison and control of the staining reactions obtained.

OBSERVATIONS

Distribution of filiform and conical papillae. The fiiliform and the conical papillae occupy the entire dorsal surface of the tongue in front of the terminal sulcus and continue on to the lateral borders. Three types of these papillae can be recognized by macroscopic and microscopic examination; because of the differences in size and shape they may be termed: *simple* and *giant* conical papillae, and *filiform* papillae (figs. 1, 2).

The greater anterior part of the tongue is beset with the *simple conical* papillae. They diminish in size and density toward the apex of the tongue. Their horny spines are strong and well curved, with the points directed backward. Their distribution is not too dense, so that interpapillary fields of the mucous membrane may easily be discerned (figs. 1, 2, 3A).

The posterior part of the body of the tongue is characterized by the presence of the true *filiform* paillae. Their horny epithelial sheaths seem to end in a few tuft-like filaments curved backward, but much thinner and less rigid than those of the simple papillae. They are arranged very densely so that, especially in the sagittal sections, interpapillary fields are almost entirely obliterated (figs. 1, 2, 3B).

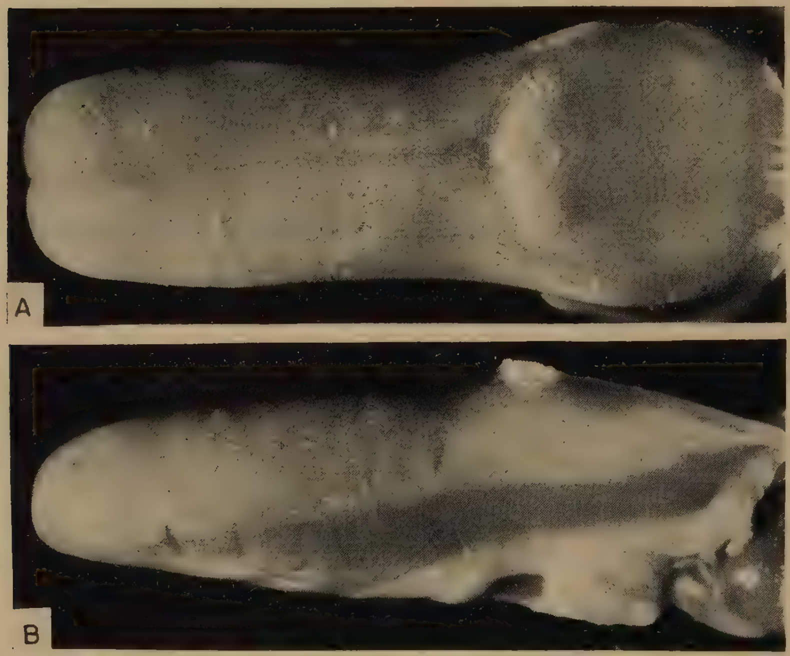


Fig. 1 Dorsal (A) and lateral (B) views of a rat's tongue. Note the elevated oval field of the giant conical papillae. Anteriorly to this field are the simple conical papillae, posteriorly the filiform papillae. × 5.

An oval field between the areas carrying the simple conical and the filiform papillae is distinguished by its greyish-white color and forms a well bordered prominence. This area carries the *giant conical* papillae. Their strong cone-shaped horny tips point in the center of the field upward. The anterior giant papillae curve backward, the posterior papillae are bent forward. On the posterior edge of the oval field, where it merges with the field of the true filiform papillae, occasional giant papillae appear to have two-pronged horny spines (figs. 2, 3B).

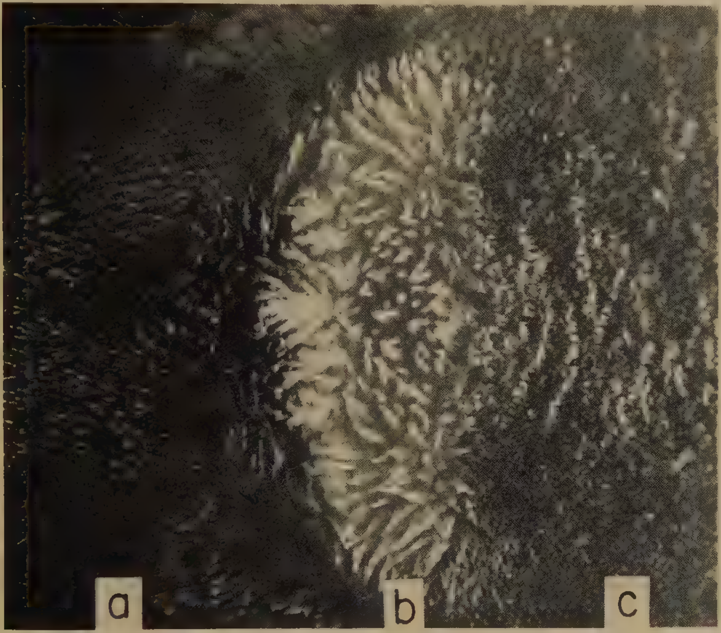


Fig. 2 Higher magnification of the lingual prominence. The three types of papillae (a, simple conical papillae; b, giant conical papillae; c, filiform papillae) are well visible. × 12.

Simple conical papilla. The simple conical papilla is characterized by a connective tissue core in the shape of a truncated cone which does not carry secondary papillae. Its epithelial covering consists of the basal layer of low columnar cells, arranged upon the basement membrane, and a thin cone of prickle cells separated by very narrow intercellular spaces and connected by a few intercellular bridges. The differentiation of these central cells takes a different course on the anterior convex and the posterior concave surfaces (fig. 4A).

Anteriorly, that is, at the base of the convexity of the backward curved papilla, the prickle cell layer is covered by 4 to 5 layers of flat cells with rather indistinct nuclei. Their cytoplasm is filled with small round granules of oily appearance.

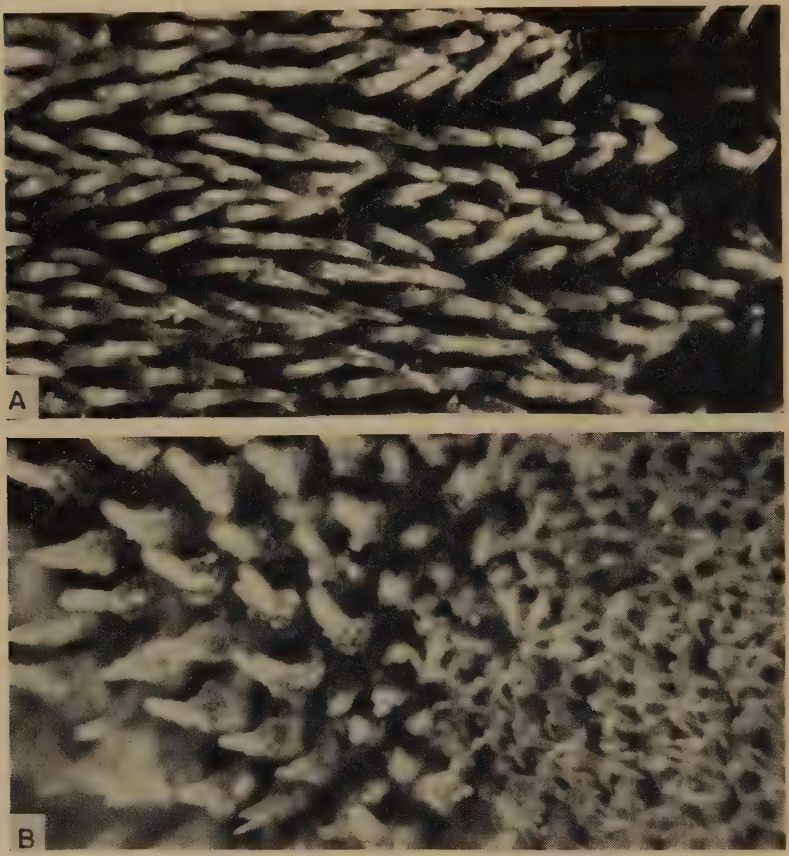


Fig. 3 The lingual papillae in high magnification. A, Simple conical papillae. B, Giant conical papillae and the filiform papillae. × 50.

They stain greyish-blue with hematoxylin-eosin, orange with Carlier's (1893) hematoxylin-benzopurpurin stain, and dark pink with Martinotti's ('24) crystal violet-eosin B stain, indicating that they are eleidin or pre-eleidin in nature.

The granular layer of cells is followed by a very thin, slightly basophilic layer (stratum lucidum) which in turn is covered by numerous layers of horny scales. The reaction with the crystal violet-eosin B stain showed them to be eleidin and post-eleidin respectively with a few keratinogenic scales on the outermost surface of the papilla. The stratum corneum of the anterior portion of the papilla reaches somewhat beyond the surface of the interpapillary fields, along the convex surface of the horny tip and could be considered as the supporting buttress of the papillary spine (fig. 4A).

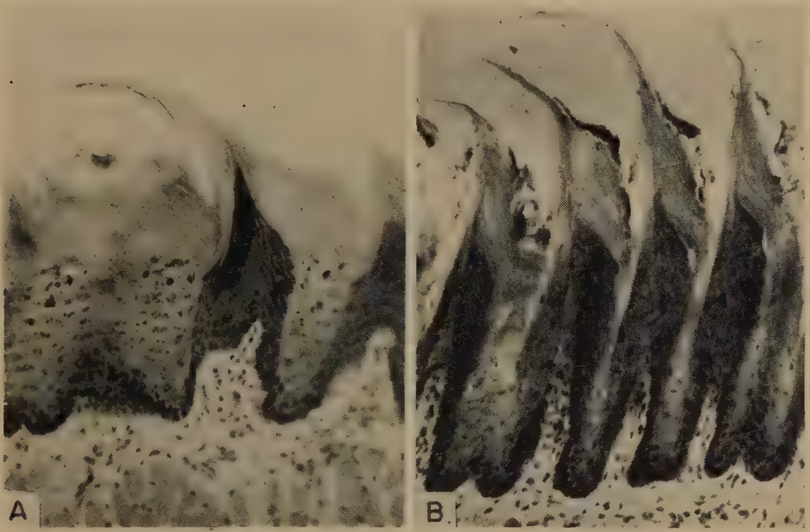


Fig. 4 Photomicrograph of lingual papillae in sagittal section. A, Simple conical papilla. B, Four filiform papillae. × 165.

Toward the posterior surface of the papilla the central prickle cells differentiate into spindle-shaped cells whose cytoplasm gains a more basophilic quality. At the same time the nuclei of these cells become pyknotic and finally disappear. On the posterior concave side of the papilla a stratum granulosum is missing. The prominent claw-like part of the papilla consists of densely packed horny scales or spindles that remain almost unstained in hematoxylin-eosin. In many of the horny scales fine granules can be seen, the nature of which cannot be determined. This evidently most solid or rigid part of the papilla forms the long and slender spine, the point of which curves backward (fig. 4A).

Hematoxylin-benzopurpurin stain showed the epithelial cells underlying the horny spine to contain diffused eleidin and the spine itself to be true keratin. Buzzi's picric acid-nigrosin stain, as described by Unna ('28), confirmed the spine to be horn. The crystal violet-eosin B stain, however, showed that the epithelial layer contains diffused eleidin, pre- and post-eleidin and that also the spine contains a fair amount of eleidin.

In the interpapillary fields the epithelium forms ridges separating the dermal cores of the papillae. The ridges consist, adjacent to the basal layer, of a few layers of irregular prickle cells continuing into the stratum granulosum. The cells of this layer are large, irregularly polyhedral with large vesicular nuclei containing a prominent nucleolus. The basophil granules are of varied size and shape. The cells of the granular layer flatten out as they continue toward the surface into a thin stratum lucidum which in turn is covered by a thick, clumpy stratum corneum (fig. 4A).

When stained with hematoxylin-eosin, the granules of the stratum granulosum of the interpapillary fields appear irregular in shape and blue-black similar to the keratohyalin granules of human skin. Crystal violet-eosin B stain, however, made them appear round, dark blue, with refractive red centers. This stain showed that the stratum lucidum of the interpapillary fields corresponds to Martinotti's post-eleidin or keratogenetic layer. The surface layers are keratinous. Picric acid-nigrosin stain reveals the presence of eleidin in the stratum corneum.

Giant conical papilla. The giant conical papilla has a dermal core that represents roughly truncated cone. It is slightly compressed in an anteroposterior direction. The surface corresponding to the convexity of the horny tip is slightly convex mediolaterally and the opposite surface slighty concave. The crest of the dermal papilla carries several low secondary papillae which continue as small ridges along the concave side of the dermal core. The epithelial structure of the giant conical papilla does not differ qualitatively from that of the simple conical papilla. The epithelium of the interpapillary fields in the region of the giant papillae is the same as that of the interpapillary fields of the anterior portion of the tongue, but the width of the epithelial ridges is greatly reduced with the increase in diameter of the dermal papillae (fig. 5).

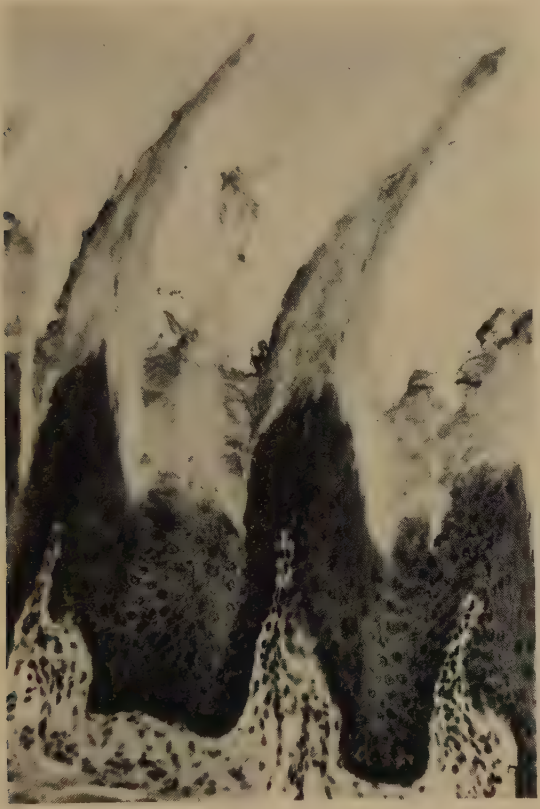


Fig. 5 Photomicrograph of two giant conical papillae in sagittal section. × 165.

Filiform papilla. The connective tissue core of the filiform papilla can be likened to a hand with fingers. It forms a plate placed in a frontal plane, from the crest of which rise 4 or 5 slender secondary papillae (fig. 6). The epithelium of the filiform papilla does not fundamentally differ from that of the conical papilla. The central prickle cells of the anterior portion of the papilla follow the same course of differentiation as those of the simple conical papilla. They form a common epithelial sheath for all of the secondary papillae and produce a single scaly elevation above the general surface of the tongue. On the posterior surface, however, the central prickle cells differentiate independently for each secondary papilla and produce several very thin horny threads, giving to the tip of the filiform papilla a tuft-like appearance. The interpapillary fields in the region of the filiform papillae are,

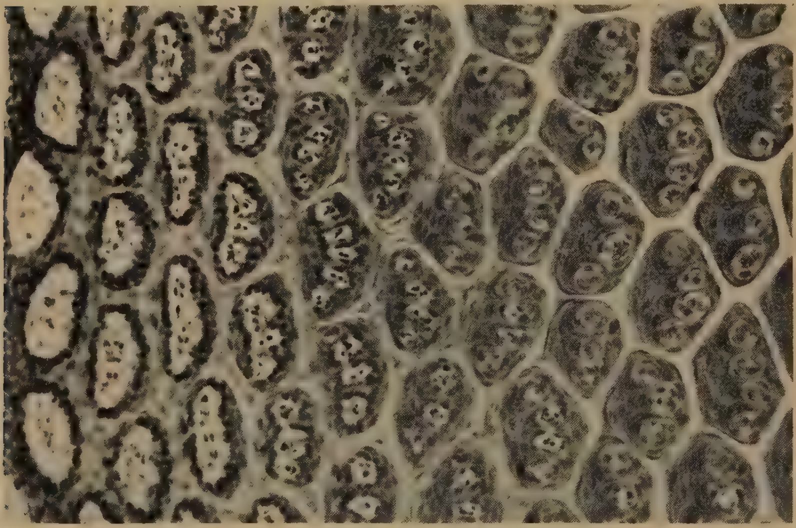


Fig. 6 Horizontal section through the posterior region of a rat's tongue. Note the cross-sections through the filiform papillae at different levels shwing from left to right the common stock and the finger-like extensions of the dermal papilla, and the bases of the horny threads. × 165.

as was mentioned before, almost entirely obliterated. (figs. 4B, 6).

DISCUSSION

A careful microscopic examination of the dorsal surface of a rat tongue revealed a peculiar differentiation of the filiform or cone-shaped papillae. Beyond remarking on the large size of the cone-shaped papillae of the area of the lingual prominence (Sonntag, '24; and Fish, Malone, and Richter, '44), no details seem to be described in the literature. It is, however, obvious that the papillae on the dorsal surface of the body of the tongue are entirely different in three distinct fields. The larger anterior area in front of the intermolar lingual prominence is beset with papillae of a simple basic pattern. Their main characteristic is the single crescent-shaped horny tip pointing backward. In this pattern they resemble closely cone-shaped papillae described on the tongue of other animals.

The papillae that are found on the lingual prominence, however, cannot be described as merely being larger cone-shaped papillae. Not only is the dermal papilla larger but also much more complicated and the horny spine shows only a slight curvature as compared with the crescent or sickle-shaped spine of the simple conical papilla. Furthermore, there is a peculiar change in the direction of the spine: those in the middle of the lingual prominence point almost straight upward, those in the anterior and posterior parts bend toward the center.

In contradistinction to the simple and the giant conical papillae the papillae behind the intermolar fields are true filiform papillae. Their horny extensions are threads rather than spines. Each one of the filiform papillae is a fairly complicated structure, because it consists of a common base compressed in the anteroposterior direction and 3 to 5 secondary papillae each of which ends in a long soft horny filament.

It is obvious that the clear differentiation of three areas on the tongue each carrying a different type of papillae must have some functional significance. No explanation of such functional correlation can be offered at this time, but a study of the mucous membrane of the hard palate may enable us to analyze the functional implications.

Histologic examination of the different papillae has not only enlarged our knowledge of their typical characteristics but has also given some information on the difficult problem of hornification. Application of series of specific stains has shown not only that the horny layer evidently consists of different types of keratin but also that there exist in the tongue different types of keratinization or hornification. Three types of keratin can be differentiated: (1) the stratum corneum of the interpapillary fields, (2) the horny substance of the spines of the cone-shaped and the threads of the filiform papillae, (3) the horny layer that covers the base of the horny spines.

The process of keratinization is either that commonly described in which a stratum granulosum and lucidum are present, or an ''abbreviated'' process without presence of granular and lucid layers. It also appears that where stratum lucidum is present the eleidin is not totally changed to keratin, but the stratum corneum contains a fair amount of latent eleidin. The findings on keratinization confirm the findings of Carlier (1893), Martinotti ('21), and Unna ('28). It is interesting, however, that the different areas of the surface in close approximation show significant differences in the chemistry of hornification. It is, of course, obvious that the different types of keratin are related to the differences in rigidity and resistance of the different areas. In order of increased resistance these areas are: interpapillary fields, butressing layer, and spine or thread.

SUMMARY

This study of the filiform and the conical papillae is based on a macroscopic and a microscopic examination of 21 tongues of normal albino rats, males and females, ranging in age from 10 days to 5 months.

The dorsal surface of the rat tongue can be roughly subdivided into three areas of probable functional significance:

1. The anterior portion of the body of the tongue that carries the *simple cone-shaped papillae.*
2. The oval field on the anterior portion of the lingual prominence beset with the *giant conical papillae.*
3. The posterior portion of the body of the tongue characterized by the *true filiform papillae.*

The three types of papillae can be distinguished on the basis of the shape and positioning of their respective dermal cores which in turn determine the size, shape, and direction of their horny spines or thread-like filaments.

The density of the distribution of the conical and the filiform papilae increases progressively from the tip to the base of the tongue with a subsequent decrease in the size of the respective interpapillary fields.

The mucous membrane of the dorsal surface of the rat tongue seems to demonstrate two different types of processes of keratinization, with the subsequent formation of three histologically and possibly chemically different keratins. The three types of keratin are closely correlated to the morphologic structure of the underlying tissues and suggest a definite adaptation to different types of function.

ACKNOWLEDGMENTS

The authors wish to express their sincere gratitude to Mr. William Winn for his excellent work in preparing all the photographs and the photomicrographs.

LITERATURE CITED

CARLIER, E. W. 1893 Contributions to the histology of the hedgehog (Erinaceus europaeus). J. Anat. and Physiol., *27:* 85–91.

COWDRY, E. V. 1948 Laboratory Technique in Biology and Medicine. The Williams and Wilkins Co., Baltimore, p. 86.

FISH, H. S., P. D. MALONE AND C. P. RICHTER 1944 The anatomy of the tongue of the domestic Norway rat. I. The skin of the tongue; the various papillae; their number and distribution. Anat. Rec., *89:* 429–440.

HOEPKE, H. 1927 Die Haut. In: Handbuch der mikroscopischen Anatomie des Menschen, by v. Moellendorff, W., *3:* 1–32.

MARTINOTTI, L. 1914 Recerche sulla fine struttura dell' epidermide umana normale in rapporto alla sua funzione eleidocheratinica. Arch. f. Zellforsch., *12:* 459–489, *13:* 446–459, 563–587, *15:* 377–392.

MARTINOTTI, T. 1924 Tecnica per lo studio del processo della cornifizazione della cuti. Ztschr. f. wiss. Microscopie, *41:* 20–258.

SONNTAG, C. 1924 The comparative anatomy of the tongue of the Mammalia. Proc. Zool. Soc. London, *2:* 725–743.

UNNA, P. G. 1928 Histochemie der Haut. Franze Deuticke, Leipzig und Wien. 53–65.

ZISKIN, D. E., G. STEIN, P. GROSS AND E. RUNNE 1947 Oral, gingival, and periodontal pathology induced in rats on a low pantothenic acid diet by toxic doses of zinc carbonate. Am. J. Orthodont. and Oral Surg., *33:* 407–446.

SAPONIN INDUCED COLONIZATION OF THE BONE MARROW ELEMENTS IN FOREIGN ORGANS IN RABBITS[1]

KŌSUKE ŌMURA AND BUNSUKE OSOGOE
Department of Anatomy, Yamaguchi Prefectural Medical College, Ube City, Japan

ELEVEN FIGURES

INTRODUCTION

The mode of establishment of extramedullary hematopoiesis is believed by most investigators to be an autochthonous formation through metaplasia from local fixed, mesenchymal cells rather than a colonization of myeloid cells by metastasis from the bone marrow.[2] Our recent study (Osogoe and Ōmura, '50) has demonstrated, however, that extramedullary foci of blood formation may be produced in the liver, spleen and other organs by means of intravenous injection of a large number of cellular elements of bone marrow into normal rabbits. The behavior of the injected bone marrow cells was compared with that of lymphoid cells described in other papers (Osogoe, '50; Osogoe and Hitachi, '50), and the evidence was presented that such foci of extramedullary hematopoiesis are formed as a result of selective accumulation, or colonization, of the injected bone marrow cells in these organs.

---

[1] This work was supported by Grant in Aid for Fundamental Scientific Research from the Department of Education of the Japanese Government. A preliminary report of this study was read at the 11th annual meeting of the Japanese Hematological Society in Kyoto, April, 1949 (Acta Haemotol. Jap., *12:* 12).

[2] For a discussion of the literature on this problem, readers are referred to the review by Lang ('38) and the more recent work by Schiller ('43). See also the monograph by Rohr ('40).

PRESS OF THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
Printed in the United States of America

The present work is an attempt to induce a similar colonization of the bone marrow elements in foreign organs in ways other than by intravenous injection of bone marrow cells of external origin. It is an attempt to find some agent which might induce the discharge of immature blood cells from the bone marrow into the circulating blood and their subsequent accumulation in other organs.

In a search for such an agent in the literature, we found an interesting work by Livadas ('33) who demonstrated that secondary foci of blood formation may occur in the liver and spleen within several hours after the injection of a lethal dose of saponin into rabbits. Livadas' finding indicates that a liberation of the bone marrow elements into the circulation may be induced by saponin injection, because an autochthonous formation of myeloid cells through metaplasia from local fixed cells seems hardly possible within such a short time. Another possibility can be seen from the work of Drinker, Drinker and Lund ('22) showing that in acute poisoning by saponin in rabbits, the bone marrow parenchyma is open to the blood current as a result of injury to the capillary walls. Accordingly, we employed saponin as the stimulating agent in the present study and found it to be a satisfactory agent when applied in a large dose.

MATERIAL AND METHODS

Animals used in the experiments were rabbits of both sexes weighing 2.4–3.1 kg. Saponin pure white (E. Merk, Darmstadt) was dissolved in physiologic saline in a concentration of 0.1%. In most instances, 5–7 cm^3 of the solution, or 5–7 mg of saponin, was given in a single injection into the ear vein. At this dosage the majority of the animals survived the injection and were able to recover, but in doses up to 8–10 mg the animals often died within several hours after the injection.

As a preliminary procedure, approximately 30 cm^3 of blood was withdrawn by heart puncture prior to saponin injection, because this enhanced the effects of the saponin to some extent although a single bleeding without saponin had no effect.

Blood examinations were made at intervals of 1–2 hours for the first 12 hours after injection and later at intervals of 12–24 hours. At successive intervals (1, 3, 6, 12, 24, 48, 72 hours and 5, 7, 10, 14, 21, 30 days) after injection, the animals were killed by the injection of air, and the lung, liver, spleen, bone marrow (femur and in some instances also sternum or ribs) lymph nodes (mesenteric and in some instances also popliteal nodes), kidneys and adrenals were studied in tissue sections. The usual staining by hematoxylin (Mayer's acid hemalum) and eosin after fixing in Zenker formol was sufficient for discrimination of the varying stages of maturation of immature blood cells in tissue sections, except for proerythroblasts, myeloblasts and basophilic myelocytes. For discrimination of the earliest forms or other cells unidentified in sections, imprint preparations were made from the organs to be studied and stained with Giemsa or May-Giemsa. The special hematoxylin and eosin-azur II method of Maximow to stain sections was not used, as the results were so clear-cut that it seemed unnecessary.

Differential counts of the immature blood cells contained in 1 cm^2 of sections cut 8 μ thick, or a volume of 0.8 mm^3 of organ, were made from different portions of the organs. Since a volume of 0.8 mm^3 of fixed and imbedded tissues corresponds closely to 1 mm^3 of the original fresh tissue, data from the sections are comparable with those from blood counts. When the sections were cut 6 μ thick, data obtained for 0.6 mm^3 were corrected to those for 0.8 mm^3.

RESULTS

Effects of saponin upon the blood and organs

The changes of the blood and organs were produced within 72 hours after the saponin injection. Data illustrative of these changes from blood examinations and differential cell counts of sections and imprints of the organs up to that time are represented in tables 1–4.

Peripheral blood. The red cell count and hemoglobin content decreased rapidly, reaching a minimum at the 72nd hour, and recovered gradually in about 30 days. The number of the blood leucocytes was reduced during the first few hours, then markedly raised during a period of 4 to 24 hours after the injection. These changes were due chiefly to decrease or increase in pseudoeosinophiles. After 24 hours numerous pseudoeosinophiles with toxic granulation were observed.

Erythroblasts occurred for the first time at the third or 4th hour and rapidly increased thereafter, so that by the 24th hour they often amounted to half the total number of the nucleated cells (table 1). They were greatly reduced during the next 48 hours, but a small number of normoblasts persisted for 2 weeks after injection. The erythroblasts appearing in the peripheral blood were for the most part normoblasts with a few macroblasts. A few metamyelocytes were occasionally seen, but we missed either myelocytes with large round nuclei or megakaryocytes (see table 1).

Lung. The immature blood cells, especially those of larger size such as myelocytes, macroblasts and megakaryocytes, occurred in this organ in much greater numbers than in the peripheral blood (table 2). Erythroblasts began to appear at the first hour, myelocytes at the 6th hour, and both became very numerous at the 48th hour, but thereafter they decreased rapidly. It is worthy of notice that up to the 24th hour myelocytes occurred in the lung in definitely greater numbers than in the liver and spleen. Megakarocytes appeared in great numbers at the 6th hour, but later they became much less numerous.

These immature cells were found within the lumen of the capillaries of the alveolar walls and other small blood vessels, scattered rather diffusely over the whole organ up to the 12th hour. But at the 24th and 48th hour, they were frequently grouped together as either diffuse or dense cell collections in the lumen of not only the small but also relatively large blood vessels, particularly the pulmonary arteries, and often obstructed their lumen partially or completely (fig. 1). These cell emboli were composed mostly of the large, early forms of both erythroid and myeloid series. The corresponding portions in imprints, therefore, gave a picture approaching that of the bone marrow (fig. 2). As seen in table 3, the imprints of the lung were rich in the large, early forms of blood cells. It should be noticed that at the 24th hour the

TABLE 1

Blood examinations[1] of the rabbits received 6 mg of saponin, subsequent to withdrawal of approximately 30 cm³ of blood

RABBIT NO.	BODY WT.	TIME AFTER INJ.	RBC (× 10^4)	HB	WBC	IMMATURE BLOOD CELLS[2]	
						Erythroblasts	Myelocytes
	kg			%			
S13 ♀	3.1	Initial	710	97	6,200	. .	. .
		1 hr.	585	75	3,700	. .	. .
		3 hrs.	563	67	3,880	20	. .
		6 hrs.	550	63	13,940	660	. .
S10 ♀	2.4	Initial	637	90	6,400	. .	. .
		1 hr.	480	64	3,000	. .	. .
		4 hrs.	496	52	9,770	1,030	. .
		8 hrs.	408	48	23.620	1,780	. .
		12 hrs.	350	45	25,820	4,380	. .
S 7 ♂	2.6	Initial	620	97	7,000	. .	. .
		12 hrs.	336	58	13,030	2,770	. .
		24 hrs.	324	52	8,860	9,040	. .
S 6 ♀[3]	2.4	Initial	610	97	6,000	. .	. .
		24 hrs.	364	45	3,570	3,430	120[4]
		48 hrs.	254	37	2,180	220	. .
S 9 ♂	2.8	Initial	630	84	7,960	40	. .
		24 hrs.	480	60	20,600	20,600	. .
		48 hrs.	440	57	19,200	5,400	. .
		72 hrs.	400	52	14,100	1,900	.

[1] The number of cells counted was 500 in each smear.

[2] Megakaryocytes were detected in the peripheral blood in no instances.

[3] This animal received a second injection of 2 mg of saponin at the 24th hour.

[4] Metamyelocytes.

TABLE 2

Differential cell counts of the immature blood cells per square centimeter of sections of the lung, liver, spleen and bone marrow cut 8 μ thick (0.8 mm³ of organ)

RABBIT NO.	TIME AFTER INJ.	ORGAN	MYELOCYTES				ERYTHROBLASTS		MEGAKARYOCYTES
			Pro-M.	Eos.M.	Pse.M.	Meta-M.	Macro.	Normo.	
S15	1 hr.	Lung	. .	. .	. .	. .	18	54	. .
		Liver	. .	. .	. .	. .	. .	90	. .
		Spleen	. .	. .	. .	. .	. .	69	. .
S13	6 hrs.	Lung	. .	. .	59	. .	118	1,300	472
		Liver	. .	. .	15	. .	30	675	. .
		Spleen	. .	. .	55	. .	1,100	39,800	. .
S10	12 hrs.	Lung	. .	34	102	. .	544	1,460	12
		Liver	. .	. .	58	. .	348	6,150	. .
		Spleen	. .	. .	35	. .	10,200	112,000	35
S 7	24 hrs.	Lung	47	94	423	47	1,360	6,200	. .
		Liver	. .	57	114	57	798	9,180	. .
		Spleen	. .	. .	106	. .	6,370	72,000	34
		Bone marrow	676	507	26,500	2,370	3,890	168,000	845
S 6	48 hrs.	Lung	105	560	1,050	350	1,540	6,400	70
		Liver	410	82	1,480	370	410	5,100	82
		Spleen	410	410	3,200 (246)[1]	490	9,920	66,000	82
		Bone marrow	1,040	232	8,000 (580)[1]	810	6,840	73,000	2,200
S 9	72 hrs.	Lung	. .	37	925 (110)[1]	185	444	1,700	74
		Liver	. .	44	4,310 (44)	484	132	1,890	790
		Spleen	525	700	28,000 (1,750)[1]	2,800	5,950	54,000	1,230
		Bone marrow	285	142	10,800 (280)[1]	1,420	2,700	38,600	1,700

---

[1] The number of mitoses involved.

Abbreviations: Pro-M., promyelocytes; Eos.M., eosinophilic myelocytes; Pse.M., pseudoeosinophilic myelocytes; Meta-M., metamyelocytes; Macro., macroblasts; Normo., normoblasts.

percentage of the myelocytes in the lung was distinctly higher than that either in the liver, spleen or bone marrow.

Besides immature blood cells, there occurred a transient collection of pseudoeosinophilic and eosinophilic polymorphonuclears in the lung capillaries shortly after the injection, coincident with the initial granulocytopenia of the peripheral blood.

Liver. In the liver, the accumulation of myelocytes and megakaryocytes was delayed but took place to a much greater extent than in the lung, during the period from the 48th to 72nd hour (table 2). Erythroblasts became very numerous during an interval between the 6th and 24th hour. Both myelocytes and erythroblasts occurred intravascularly, either scattered or grouped together as small cell collections, in the sinusoids between the liver cell cords. Megakaryocytes appeared singly in the lumen of the sinusoids. At the 72nd hour the gross appearance of the sections of the liver resembled that of the sections of the embryonic liver (fig. 3). By contrast in the periportal tissue spaces, neither myelocytes, erythroblasts nor collections of round cells, which were often observed in the case of intravenous injection of bone marrow (Osogoe and Omura, '50), appeared at either time interval.

The myelocytes accumulated in the liver were sometimes in mitosis (table 2), and there was no indication of their rapid degeneration up to the 5th day. The erythroblasts in the liver also showed no degenerative change until the 48th hour, although they were very much reduced during the next 24 hours. Besides myelocytes and erythroblasts, the earliest forms, myeloblasts and proerythroblasts, were also observed in the imprints of the liver but only in small numbers (table 3).

The hepatic cells showed no significant changes except a variable degree of vacuolation of the cytoplasm which recovered rapidly. The Kupffer cells were often enlarged, some assuming the shape of basophilic round cells, others in mitosis. These changes of Kupffer cells, however, manifested them-

TABLE 3

Differential cell counts[1] *(%) from imprint preparations of the lung, liver, spleen and bone marrow at the 24th and 48th hour after saponin injection*

CELL TYPE	24TH HOUR				48TH HOUR			
	Lung	Liver	Spleen	Bone marrow	Lung	Liver	Spleen	Bone marrow
Proerythroblasts	1.5	..	0.1	..	4.0	0.5	1.9	0.2
Macroblasts	5.75	2.5	1.4	1.2	12.5	6.5	6.5	2.6
Normoblasts	29.25	46.25	52.0	66.3	41.25	51.25	40.9	39.2
Myeloblasts	..	..	0.3	..	1.0	0.5	0.7	..
Promyelocytes	3.0	2.0	0.2	0.8	2.0	2.25	1.2	13.2
Myelocytes	3.5	1.0	0.3	1.7	2.25	6.25	4.0	11.5
Metamyelocytes	1.25	3.5	0.2	2.0	3.0	7.0	1.0	7.9
Pseudoeosinophiles	17.75	24.0	14.4	7.8	12.25	8.0	1.5	5.1
Eosinophiles	0.75	..	0.2	0.1	0.25	..	0.3	0.1
Basophiles	1.5	1.0	0.1	0.5	0.25	..	0.1	0.2
Lymphocytes	18.25	8.75	23.3	9.2	13.0	10.0	40.4	14.2
Monocytes	17.0	10.75	5.5	7.0	7.25	7.25	0.5	2.5
Unidentified cells	0.5	0.25	2.0	3.4	1.0	0.5	1.0	3.3

[1] The number of cells counted was 400 in the lung and liver and 1,000 in the spleen and bone marrow.

selves after the accumulation of immature blood cells in the sinusoids had almost been accomplished, and no evidence could be obtained in favor of a direct transformation of the Kupffer cells into the early forms of blood cells. In most instances, there was a slight degree of sinusoidal dilatation due to congestion with an accumulation of granulocytes shortly after the injection, but it receded rapidly.

Spleen. The immature blood cells accumulated in this organ more than in all the others organs by the 72nd hour (table 2). The organ usually became 3–5 times its normal weight

As early as 6 hours after the injection, there occurred numerous erythroblasts (both normoblasts and macroblasts) in the lumen of the venous sinuses showing a marked dilatation due to congestion. By the 12th hour the erythroblasts per unit volume of the spleen amounted to an enormous number (see table 2). Until the 24th hour they occurred almost exclusively in the dilated venous sinuses where they were densely aggregated (fig. 4), but at the 48th and 72nd hour the endothelial wall of the sinuses often disappeared partially or completely and the erythroblasts infiltrated rather diffusely over a wide area of the red pulp (fig. 5). In such instances, the contour of the venous sinuses was imperceptible and the red pulp appeared structureless.

A considerable degree of accumulation of myelocytes took place during the period from the 48th to 72nd hour, and finally their number per unit volume became greater than that in the bone marrow (see table 2). The chief accumulation of myelocytes was in the splenic cords outside of the venous sinuses, usually grouped together as either diffuse or compact cell collections (fig. 5). During the same period megakaryocytes also became very numerous. They appeared singly in the venous sinuses or other parts of the red pulp.

The immature cells accumulated in the spleen, particularly the myelocytes, showed a large number of mitoses, and there was no indication of any degenerative changes of these elements up to the 10th day. In the imprints of the spleen, myeloblasts were more frequent than in the liver (table 3).

Besides immature forms, mature leucocytes also collected in the red pulp shortly after the injection, but the majority of them underwent disintegration and disappeared by the 48th hour. Macrophages containing erythrocytes or hemosiderin granules were frequently met but in lesser numbers than had been expected. The white pulp was markedly reduced in size by the 48th hour. This is due to the disappearance of the diffuse lymphatic tissues surrounding the nodules which remained almost unchanged.

Bone marrow. The changes of the bone marrow were initiated by a marked sinusoidal dilatation due to congestion and subsequent hemorrhage shortly after the injection. The marrow hemorrhage increased in degree with lapse of time. In addition, there occurred localized liquefaction of the marrow matrix. At the 12th hour, we met here and there with irregularly shaped areas of varied sizes where erythrocytes and marrow elements of varying stages of maturation were diffusely mingled. Such areas were in direct communication with the marrow parenchyma without endothelial barrier (fig. 7).

During the interval between the 6th and 24th hour after injection, the cellularity of the bone marrow was strikingly reduced so that by the end of this interval, or at the latest by the 48th hour, the marrow parenchyma was depleted almost completely. During the same interval the marrow hemorrhage was considerably increased in degree, and the depleted marrow parenchyma was flooded with blood. Consequently, the marrow parenchyma appeared to have been replaced to a large extent by the extravasated erythrocytes (fig. 8). The capillary sinusoids embedded in the parenchyma became unrecognizable. India ink injected into the ear vein at this stage easily penetrated into the depleted marrow parenchyma.

During the course of this process, degenerating marrow cells were met not infrequently, especially at its final stage, but they were apparently in lesser numbers than might be responsible for the rapid depletion of the bone marrow.

After 48 hours the majority of the remaining marrow cells underwent disintegration and disappeared gradually. The extravasated erythrocytes also disappeared gradually, being replaced by a homogeneous material which stains slightly bluish-red with hemalum and eosin.

Mesenteric lymph nodes, kidneys and adrenals. The early forms of blood cells occurred also in these organs but in much smaller numbers than in the lung, liver and spleen (table 3). The majority of these cells were intravascular, either single or grouped together as small clusters. They were found in the postcapillary vein of the lymph nodes; in the glomerular and other capillaries or venules of the kidney; and in the sinusoids of the zona fasciculata and zona reticularis of the adrenal cortex.

In the kidneys, extravascular or subendothelial collections of myelocytes were often observed along the arcuate vein or its cortical branches at the 48th or 72nd hour after injection. The most interesting fact seen here is that all of the successive stages of the emigration of myelocytes through the vascular wall could be traced (figs. 9–11).[3] Essentially, the process is not a true emigration but a passive change of the myeloid cell collections from an intravascular to an extravascular situation by the formation of new endothelium covering the collections and the simultaneous disintegration of the original endothelium.

The cellularity of the lymph nodes was reduced by the injection of saponin but to a much lesser extent than that of the bone marrow. The majority of the cortical nodules disappeared while the medullary lymph sinuses were crowded with lymphocytes, so that the boundary between the cortex and medulla became inconspicuous. The endothelial wall of the medullary sinuses often disappeared to a greater or lesser degree, but examination of the efferent lymph from popliteal nodes showed no increased output of lymphocytes.

---

[3] A similar process was also observed in the case of intravenous injection of bone marrow and described in detail in our previous paper (Osogoe and Ōmura, '50).

TABLE 4

Differential cell counts of the immature blood cells per square centimeter of sections of the lymph nodes, kidneys and adrenals cut 8 μ thick (0.8 mm³ of organ)

RABBIT NO.	TIME AFTER INJ.	ORGAN	MYELOCYTES				ERYTHROBLASTS		MEGA-KARYO-CYTES
			Pro-M.	Eos.M.	Pse.M.	Meta-M.	Macro.	Normo.	
S14	3 hrs.	Lymph nodes [1]	..	..	..	..	..	..	..
		Kidneys	..	..	..	..	..	..	..
		Adrenals	..	..	..	..	..	..	..
S13	6 hrs.	Lymph nodes [1]	..	36	36	..	..	36	..
		Kidneys	..	36	..	..	48	240	..
		Adrenals	..	36	..	..	..	864	..
S10	12 hrs.	Lymph nodes [1]	..	..	21	..	..	126	..
		Kidneys	..	48	24	..	910	6,700	..
		Adrenals	..	25	50	25	625	7,800	..
S 7	24 hrs.	Lymph nodes [1]	..	16	48	16	..	96	..
		Kidneys	45	..	180	135	225	2,800	..
		Adrenals	23	..	69	46	160	2,300	..
S 6	48 hrs.	Lymph nodes [1]	..	150	210	150	..	1,770	..
		Kidneys	110	22	240	55	88	2,900	..
		Adrenals	16	48	144	110	96	1,840	..
S 9	72 hrs.	Lymph nodes [1]	15	60	45	15	..	135	..
		Kidneys	42	..	84	42	..	2,350	42
		Adrenals	..	..	95	19	38	135	..

[1] Mesenteric lymph nodes.

Abbreviations: Pro-M., promyelocytes; Eos.M. and Pse.M., eosinophilic and pseudoeosinophilic myelocytes; Meta-M., metamyelocytes; Macro., macroblasts; Normo., normoblasts.

Subsequent fate of the immature blood cells accumulated in the liver, spleen and other organs

After 72 hours the immature blood cells in the lung,[4] lymph nodes,[5] kidneys and adrenals decreased rapidly in number and disappeared almost completely by the 7th day, but in the liver they persisted for 10 days, and in the spleen for three weeks.

---

[4] In the lung a few megakarocytes persisted for three weeks after injection.

[5] In two examples killed two weeks after injection, myelocytes occurred in the medullary cords of the mesenteric lymph nodes in great numbers (760–1,250 per cm² of section).

In the liver, their number did increase rather than decrease at the 5th day, but they were gradually reduced during the next 5 days, disappearing completely on the 14th day. In the spleen, the erythroblasts which infiltrated rather diffusely over a wide area of the red pulp became aggregated again in the venous sinuses at the 5th day. By that time the endothelial wall of the sinuses, which had disappeared partially or completely, was reformed. Consequently, the erythroblasts collections in the venous sinuses were distinctly separated from the myelocyte collections in the splenic cords (fig. 6). At this stage myelocytes, erythroblasts and megakaryocytes were much more numerous than at the 72nd hour, and no marked reduction in these elements was recognized until the 10th day. They were gradually reduced during the next 11 days, and were no longer seen anywhere on the 30th day. Regeneration of the white pulp took place during the period from the 7th to 14th day.

The bone marrow parenchyma, which was depleted to a large extent by the 24th or 48th hour, began to regenerate at the 7th day and returned gradually to the normal in about 30 days. At the same time, the blood picture also recovered almost completely, except for a somewhat lower hemoglobin content (62–67%).

DISCUSSION

The present experiments have shown that a striking accumulation of immature blood cells may be produced in the sinusoids of the liver and the red pulp of the spleen and to a lesser extent also in the small blood vessels of lungs, kidneys, adrenals and lymph nodes in less than 72 hours after intravenous injection of a single large dose of saponin into rabbits. The following observations indicate that it takes place as a result of colonization of immature blood cells discharged from the bone marrow by hematogenous metastasis: (1) The output of numerous erythroblasts into the peripheral blood began to occur soon after the injection; (2) the occurrence of a great number of erythroblasts in the venous sinuses of the splenic pulp as early as 6 hours after the injection; (3) the abundant occurrence of emboli of the large, early forms of blood cells, especially the myelocytes, in the small blood vessels of the lung in the early period; (4) the rapid depletion of the bone marrow parenchyma without corresponding disintegration of immature cells therein.

Comparison of the present results with the previous observations (Osogoe and Ōmura, '50) on the behavior of the bone marrow cells transplanted into the circulating blood is of particular interest. When injected in large amounts into the ear vein of normal rabbits, the bone marrow cells, especially those of larger size such as myelocytes and megarkaryocytes, first became blocked in the lung capillaries, and later, during the period from the 48th to 72nd hour after injection, they were gradually transported to the liver, spleen and other organs where they accumulated, without being detected in the peripheral blood. A similar sequence of events could be traced in the present experiments. As seen in tables 2 and 3, myelocytes occurred in the lung as cell emboli in definitely greater numbers than in the liver and spleen up to the 24th hour after saponin injection. Likewise more numerous megakaryocytes were met in the lung than in the latter organs at the 6th hour. After 24 hours the circumstances were gradually reversed, and at the 72nd hour these elements accumulated in the liver and spleen to a much greater extent than in the lung. It is therefore evident that the myelocytes and megakaryocytes discharged into the circulation were at first arrested by the lung capillaries, and later they were gradually transported with arterial blood to the liver and spleen where they accumulate. During the course of this process, however, as in the previous experiments, neither myelocytes nor megakaryocytes were observed in the peripheral blood. That the absence of these cells in the peripheral blood does not favor their autochthonous origin in the sites where they accumulate has been emphasized in our previous publication.

In both experiments, the erythroblasts were deposited preferably in venous sinuses and other small blood vessels where the blood stagnates, while the myelocytes tended to migrate outside of the vessels. In the spleen, the myelocytes accumulated in the splenic cords outside of the venous sinuses. In the kidney, small perivenous collections of myelocytes were often observed along the arcuate vein and its cortical branches where all of the successive stages of the emigration of myelocytes through the vascular wall could be traced (figs. 9–11). The significance of the latter process in the histogenesis of foci of extramedullary hematopoiesis has been discussed in our previous paper.

Quantitatively, there are differences between both experiments, however. Cells of smaller size such as normoblasts appeared after saponin injection in the peripheral circulation in much greater numbers than after transplantation. Furthermore, immature cells of both large and small types accumulated after saponin injection in the lung, liver and spleen more abundantly than after transplantation. This was true even when marrows of all the long bones from 7 adult rabbits were given at a time into one rabbit. These facts indicate that more uninjured marrow cells are discharged by saponin injection into the circulating blood than can be introduced by intravenous injection of marrow cells. As noted in the previous paper, the majority of the marrow cells, especially the erythroblasts, were damaged to a greater or lesser degree by the procedure of making cell suspension, and hence they underwent regressive change rapidly upon injection. For this reason, a great number of degenerating erythroblasts stagnated in the portal system of the liver and often formed periportal round cell collections. Saponin, on the other hand, seems to be less injurious to these cells although applied in a large dose. It should be emphasized that in the present experiments there was no indication of accumulation of degenerating erythroblasts either in the portal system or periportal areas of the liver. Thus the use of saponin is a more favorable method than the intravenous injection of cellular elements for the transfer of bone marrow cells to foreign organs.

As to the mechanism whereby the marrow cells are discharged into the blood current, an injury to the endothelial wall of the capillary sinusoids resulting in extensive marrow hemorrhage must first be considered. The marrow hemorrhage began to occur shortly after saponin injection and reached a maximum at the 24th or 48th hour, by which time the marrow parenchyma was depleted almost completely, being flooded with blood. Thus the depletion of the marrow elements proceeds parallel to the marrow hemorrhage. As stated in the introduction, Drinker et al. ('22), who used the methods of perfusion and injection, showed that in acute saponin poisoning in rabbits, there is an opening of the marrow parenchyma to the blood current due to an injury to the capillary walls. By injection of India ink into the ear vein, we also confirmed that the dye easily penetrates into the depleted marrow parenchyma. A similar injury to the endothelial walls of the venous sinuses of the spleen and the medullary sinuses of the lymph nodes were also observed. Secondly, attention should be given to the localized liquefaction of marrow matrix which took place prior to the depletion of bone marrow, for marrow cells must first be freed from the stroma before they enter the blood current. According to Isaacs ('30) and Miyake ('50), hemorrhage or other intensive stimulus to blood regeneration induces the rapid liquefaction of comparatively large areas of marrow matrix. It is conceivable, therefore, that the preliminary bleeding in the present experiments might contribute to the liquefaction of marrow matrix. Probably this is the reason why it enhanced the effect of saponin.

In the present experiments, the immature blood cells accumulated in the liver and spleen, especially the latter, persisted for a much longer period than in the previous experiments. This is undoubtedly due to a marked insufficiency of the hematopoietic function of the depleted bone marrow.

SUMMARY

1. A sublethal dose of saponin was administered in a single, intravenous injection into adult rabbits, subsequent to bleeding of approximately 30 cm^3 of blood.

2. In less than 72 hours after injection, a striking accumulation of immature blood cells took place in the sinusoids of the liver and the red pulp of the spleen and to a lesser extent also in the small blood vessels of lungs, kidneys, adrenals and lymph nodes, with the formation of numerous hematopoietic foci.

3. The bone marrow parenchyma, on the other hand, was depleted almost completely and flooded with blood by the 24th or 48th hour, without corresponding disintegration of immature cells therein.

4. The general characteristics of the manner of accumulation of immature blood cells in the organs are the same as observed for the bone marrow elements which were brought in large numbers into the circulating blood of normal rabbits.

5. Evidence was presented that the use of saponin is a more favorable method than the intravenous injection of cellular elements for the transfer of bone marrow cells to foreign organs.

6. The accumulated immature cells in the spleen persisted for three weeks, while those in other organs disappeared to a large extent within 7 to 10 days after injection.

LITERATURE CITED

DRINKER, C. K., K. R. DRINKER AND C. C. LUND 1922 The circulation in the mammalian bone-marrow. Am. J. Physiol., *62:* 1–92.

ISAACS, R. 1930 The physiologic histology of bone marrow. The mechanism of the development of blood cells and their liberation into the peripheral circulation. Folia haematol., *40:* 395–405.

LANG, F. J. 1938 Myeloid metaplasia. Downey's Handbook of Hematology. Hoeber, New York, *3:* 2103–2144.

LIVADAS, K. 1933 Über myeloid Herdbildung. Folia haematol., *49:* 388–401.

MIYAKE, H. 1950 Pathology of leukemoid reaction. Tokyo Med. J., 67, *No. 8:* 1–5. (Japanese.)

OSOGOE, B. 1950 Transplantation of hematopoietic tissue into the circulating blood. I. Experiments with lymph nodes in normal rabbits. Anat. Rec., *107:* 193–220.

OSOGOE, B., AND T. HITACHI 1950 Über die Lymphocytennatur der Thymocyten. (Das Verhalten der in die Blutbahn transplantierten Thymocyten im Vergleich mit dem der Lymphocyten.) Okajimas Folia Anat. Jap., *23:* 51–56.

OSOGOE, B., AND K. ŌMURA 1950 Transplantation of hematopoietic tissues into the circulating blood. II. Injection of bone marrow into normal rabbits, with special reference to the histogenesis of extramedullary foci of hematopoiesis. Anat. Rec., *108:* 663–686.

ROHR, K. 1940 Das menschliche Knochenmark. Georg Thieme, Leipzig.

SCHILLER, W. 1943 Local myelopoiesis in myeloid leukemia. Am. J. Path., *19:* 809–837.

EXPLANATION OF PLATES

All of the photomicrographs except figure 2 were taken from preparations fixed in Zenker formol and stained with Mayer's acid hemalum and eosin.

PLATE 1

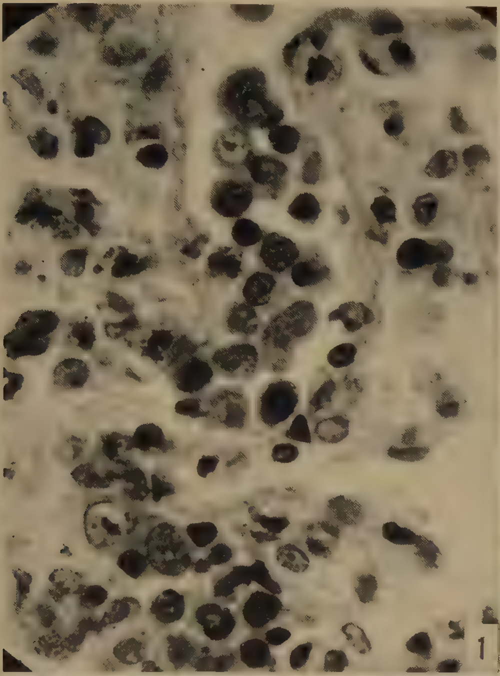
EXPLANATION OF FIGURES

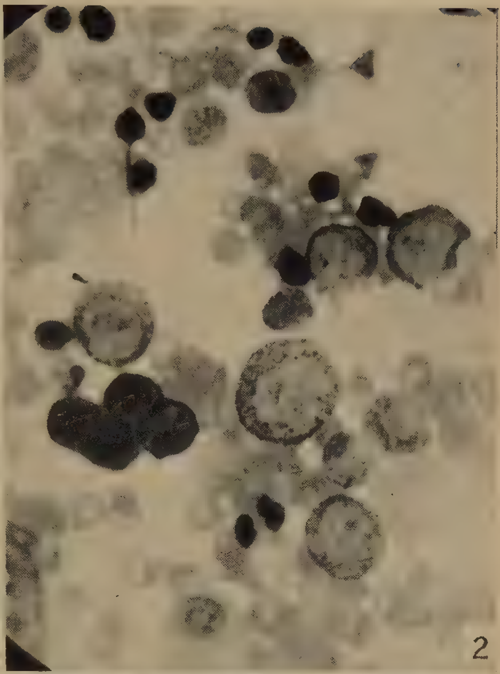
1 Section through the lung illustrating a myeloid cell embolus in the small pulmonary vessel; 24 hours after saponin injection. × 800.

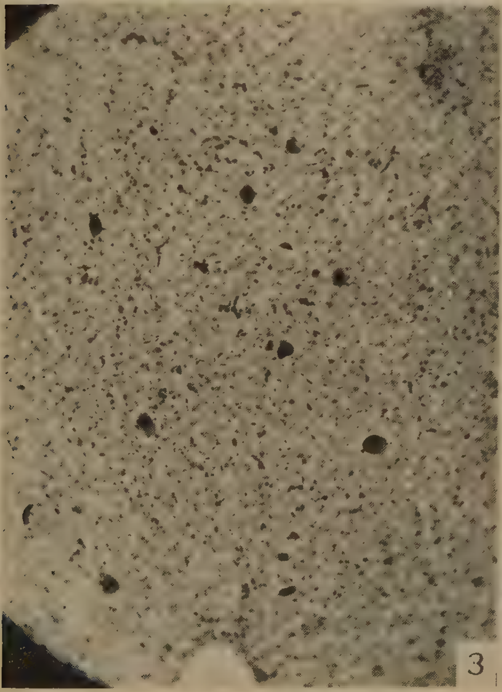
2 A collection of myelocytes and erythroblasts in a portion of the lung imprint, probably corresponding to a myeloid cell embolus; 24 hours after saponin injection. × 800.

3 Low power view of section from the liver, showing numerous small hematopoietic foci and many megakaryocytes; 72 hours after saponin injection. × 90.

4 Dense aggregations of erythroblasts in the dilated lumen of the venous sinuses of the splenic pulp. Masses of nuclear debris of degenerating granulocytes are seen in these erythroblast collections and in the splenic cords; 24 hours after saponin injection. × 400.


1


2


3

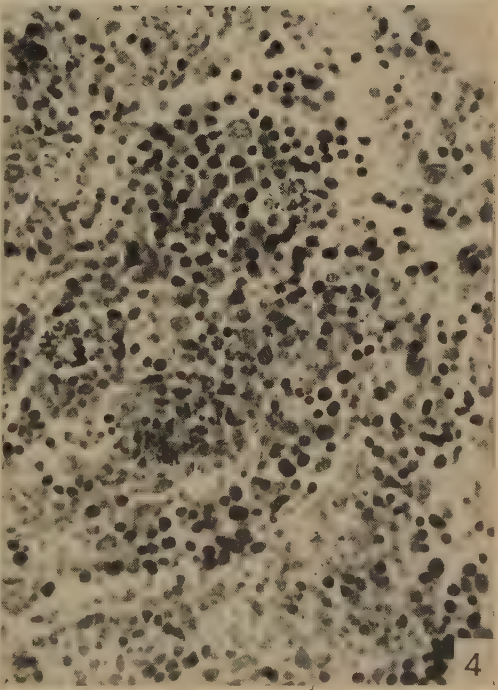

4

PLATE 2

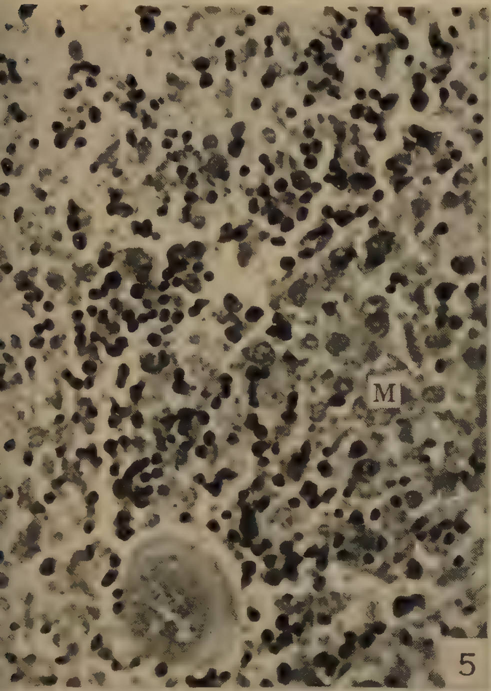
EXPLANATION OF FIGURES

5 Section through the spleen, illustrating a large myelocyte collection (M) in the splenic cord. The endothelial wall of the venous sinus containing a megakaryocyte is partially destroyed and many erythroblasts are infiltrating into the splenic cords; 72 hours after saponin injection. × 400.

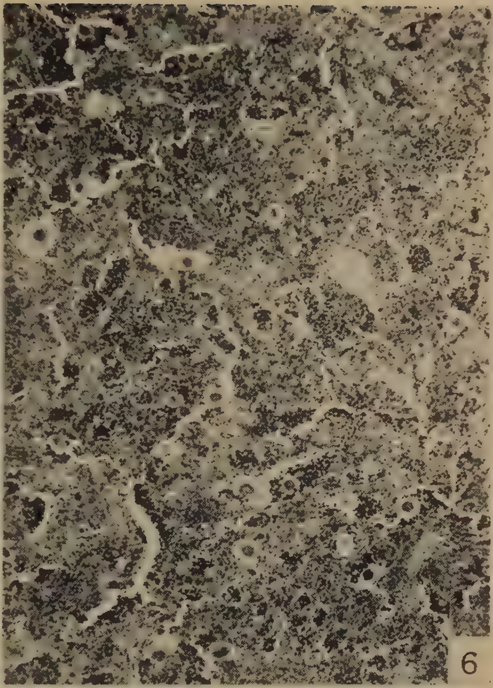
6 Low power view of section from the spleen, showing numerous, dense erythroblast collections and many megakaryocytes. The erythroblast collections are in the sharply demarcated venous sinuses, while the myelocyte collections, not visible in the figure, are in the splenic cords. Fifth day after saponin injection. × 80.

7 A relatively large area of the bone marrow where the marrow matrix is liquefied; 12 hours after saponin injection. × 80.

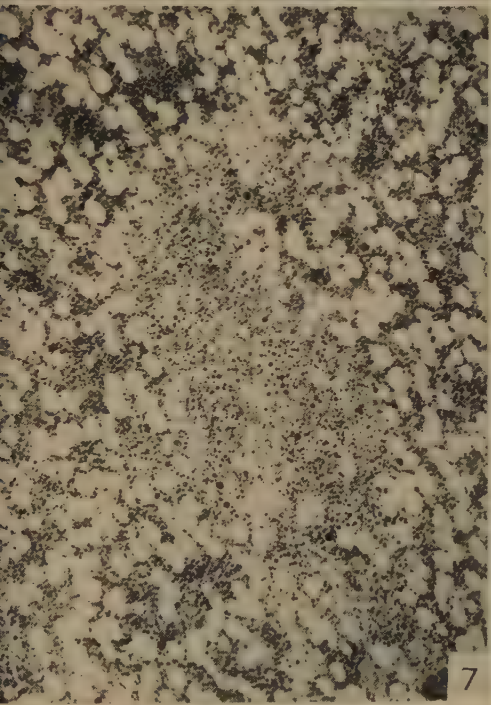
8 Section through the depleted bone marrow. Notice extensive marrow hemorrhage, with a few nucleated cells; 24 hours after saponin injection.. × 360.



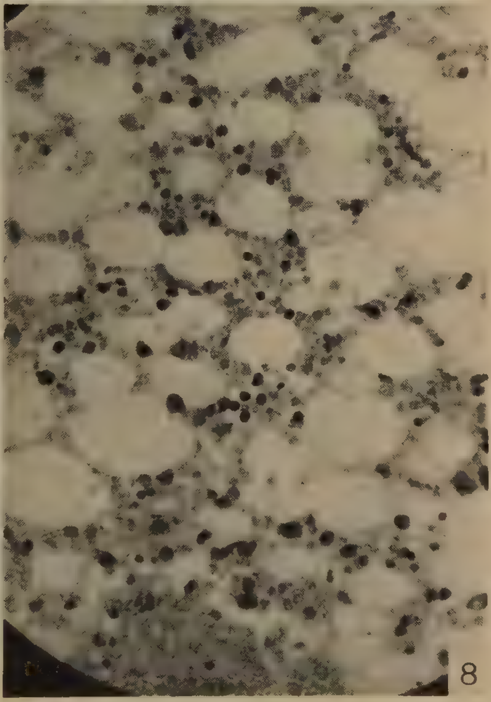
5



6



7



8

PLATE 3

EXPLANATION OF FIGURES

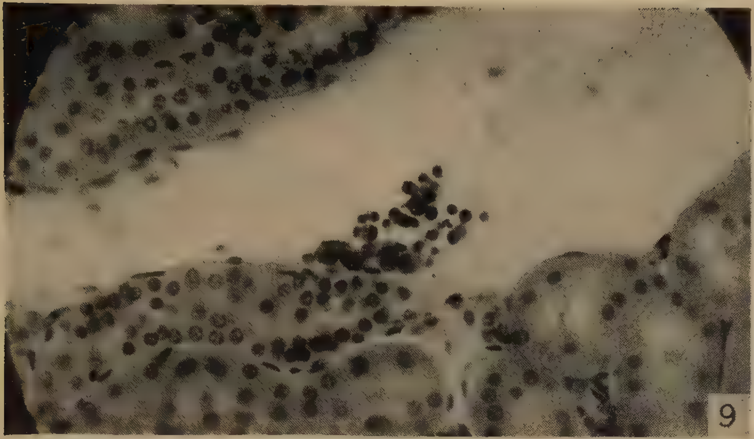
Figures 9–10 illustrate successive stages of the process of the change of myeloid cell collections from an intravascular to an extravascular situation by the formation of new endothelium covering the collections and the disintegration of the original endothelium.

9 An intravascular myeloid cell collection adheres to the inner surface of the wall of the interlobular vein of the kidney. Notice 4 large myelocytes attached closely to the endothelium; 72 hours after saponin injection. × 360.

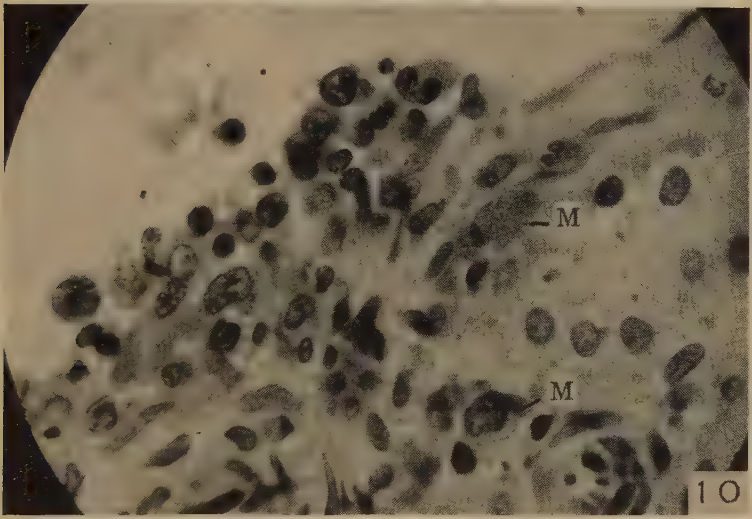
10 High power magnification of a myelocyte collection on the wall of the interlobular vein of the same kidney as in figure 9, being intermediate in position from the inside to the outside of the vessel. Notice a thin layer of fibrin threads covering the free surface of the collection and the complete disappearance of the original endothelium to which the collection adhered. Two myelocytes (M) are wandering deeply into the perivenous connective tissue. × 800.

11 A subendothelial myelocyte collection on the wall of the interlobular vein of the same kidney as in figures 9–10. Notice the newly formed endothelium completely covering the free surface of the collection and the disappearance of the original endothelium to which the collection adhered. The myelocytes do not infiltrate into the adjacent tissue, probably because of the narrow, perivenous connective tissue space. On the opposite side of the vessel, a naked erythroblast collection adhering to the endothelium is seen. × 360.

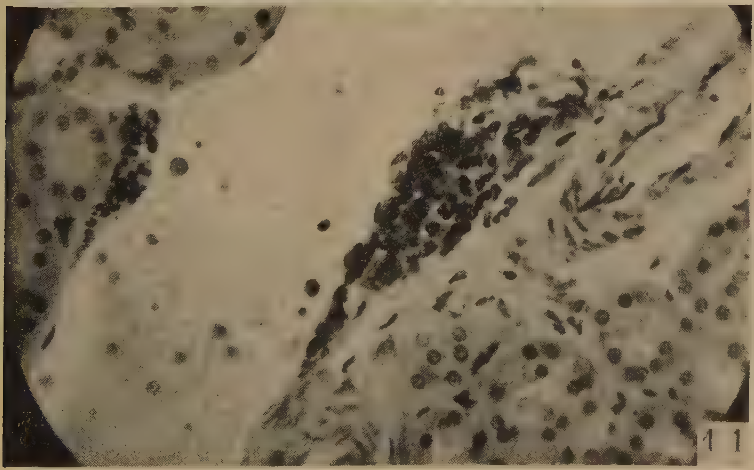
PLATE 3



9



10



11

HISTOPATHOLOGY OF AMINO ACID DEFICIENCIES

I. PHENYLALANINE [1]

CHARLES SCHWARTZ, EARL B. SCOTT AND RALPH L. FERGUSON [2]
Departments of Biochemistry, Anatomy, and Pathology, University of South Dakota School of Medicine, Vermillion

TWENTY-SEVEN FIGURES

INTRODUCTION

The consequences of protein and amino acid deficiencies are in their general aspects well known, but as pointed out by Cannon ('48) the existence of specific pathological lesions characteristic of a deficiency of a single indispensable amino acid from an otherwise complete diet has not yet been clearly demonstrated. The work reported in this paper, which is the first of a series to be presented, arose from an attempt to determine whether or not such specific results of amino acid deficiencies do exist. The results of phenylalanine deficiency will be presented. The only preceding work, from a histological point of view on the results of a deficiency of this amino acid has been presented by Maun, Cahill and Davis ('45a), but since the diet used by them was somewhat different from the one used in this work, and since the observations reported here differ somewhat from theirs, and include observations on tissues not reported by them, it seemed worth while to present these results at the present time. Further reference to the work of these investigators will be made in the discussion.

---

[1] This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

[2] Present address: St. John's Hospital, Joplin, Missouri.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain 25–30 days old were placed on the complete diet containing 19 amino acids [3] as described by Rose, Oesterling and Womack ('48). Rats of a similar age were placed on the same diet lacking the essential amino acid phenylalanine, on a diet lacking phenylalanine and tyrosine, and on a diet lacking only tyrosine. The diets were fed *ad libitum,* and the rats weighed thrice weekly. At 42 days, when typical symptoms of protein deficiency had developed, rats from each group were sacrificed and some of the rats on the deficient diets were placed on a complete diet. These latter rats were sacrificed about 55 days after having been placed on the complete recovery deit. Another group of animals was fed the complete diet in limited quantities so as to check the results of insufficient intake.

The rats were sacrificed by exsanguination under ether anesthesia. The following tissues were removed for histlogical examination: pituitary, lung, brain, thyroid, skeletal muscle, heart, thymus, liver, spleen, intestine, pancreas, adrenals, kidney, testes, epididymides, ventral prostate, seminal vesicles, and tibia.

RESULTS

The gross symptoms of protein deficiency as described by others (weight loss, loss of appetite, unthrifty appearance), developed rapidly. During the experimental period in which the control rats attained an average weight of 155 gm from an initial average weight of 45 gm, the deficient rats lost an average of 6 gm from their average initial weight of 46 gm. Of this group of deficient rats, those to which a complete diet was given after the experimental period indicated above, appeared to resume normal growth and reached an average weight of 164 gm at the time they were sacrificed. Organ weights in terms of percentage of body weight were variable, but it is hoped that a further report will deal at length with that aspect of this work on amino acid deficiencies.

---

[3] We are indebted to the Dow Chemical Company of Midland, Michigan for a few of the amino acids used in the preliminary phases of this work.

In so far as the results reported here are concerned, it can be stated that a dietary deficiency of tyrosine alone had no apparent effect on any of the tissues examined or on the general health and appearance of the animals, and in all respects the animals of the tyrosine deficient group were found to be normal. For purposes of simplification, since all rats deficient in either phenylalanine alone or phenylalanine and tyrosine together responded similarly, the changes found will be described on the basis of phenylalanine deficiency. Of all structures examined, only those described below were observed to have been affected histologically. Alteration or modifications of other tissues, other than size, if they occurred, could not be detected by the methods used.

Testes and accessory structures. Those rats fed on a phenylalanine deficient diet developed marked atrophy of the testes, epididymides, ventral prostate and seminal vesicles. The seminiferous tubules (fig. 2) were extensively degenerated, many being composed mainly of Sertoli cells resting on a basement membrane. In the tubules not so extensively damaged, spermatogenic activity appeared to have ceased at the early spermatocyte stage, with many of these cells sloughed off into the lumina and becoming karyolytic. Large numbers of these free atrophic cells were markedly eosinophilic. Mitotic figures were occasionally found in the most peripheral row of germ cells, but in the main, spermatogenesis advanced no further than the spermatocyte stage. Single and multinucleated giant cells were in evidence in the lumina of the tubules. No spermatozoa were present in the testes or epididymides although the latter contained many of the atrophic cells freed from the seminiferous tubules.

Testicular interstitial tissue was present but the Leydig cells were altered morphologically and resembled fibrocytes (fig. 8). Many of the nuclei of these cells had become elongated, rather than round or oval as is seen in normal testes, and the cytoplasm was greatly reduced in quantity, resulting in spindle-shaped cells.

The atrophic state of the epididymides, ventral prostate (fig. 11), and seminal vesicles (fig. 5) presented evidence that the testicular interstitial tissue was functionally inactive. The epididymal epithelial cells were crowded and the entire structure atrophic. The prostatic acini were very much reduced in size with marked epithelial regression. These cells were reduced in height and had lost the characteristic light area occupied by the Golgi body (fig. 14). Likewise, the epithelium of the seminal vesicles had undergone severe involution. The prostate and seminal vesicles resembled those seen in surgically castrated rats.

Adrenal glands. Of the 10 rats deficient in phenylalanine, 8 developed atrophy of the zona glomerulosa of the adrenal cortex (fig. 17). The entire gland was smaller than normal due to the size of the animals, but except for the distinct alteration of the glomerulosa, the remainder of the cortex was normal in appearance. The glomerulosa cells were compact and poor in cytoplasm with condensation of nuclear material. The medullary area was unaltered. The adrenals of two of the deficient rats did not differ histologically from the normal.

Thyroid gland. Change in the thyroid glands of the phenylalanine deficient rats was present but not strikingly distinct. The follicles were reduced but variable in size from large colloid filled follicles to extremely small ones, many without colloid (fig. 20). The follicular cells were normal morphologically although their height appeared to be slightly less than normal.

Thymus. The experimental rats were examined at autopsy and it was found that the thymus glands of all phenylalanine deficient animals were extremely atrophic and, in most cases, unidentifiable. No attempt was made to dissect out and study this structure histologically.

Pituitary gland. The pars anterior appeared to be the only portion of the pituitary affected by the absence of phenylalanine from the diet. The acidophils were conspicuously altered, being very much reduced in size and staining intensity (fig. 23). They apparently were reverting to a chromophobic state, having only a thin ring of faintly red-staining cytoplasm remaining. The basophils were fainter and fewer than normal, but not markedly smaller. No enlarged basophils or cells resembling castrate ''signet-ring'' cells were present.

Bone. Somatic growth, as demonstrated by the growth of the epiphyseal cartilage plate of the tibia, was greatly reduced. The plate was thinner than in the normal rats and bony trabeculae were strikingly absent particularly in the diaphysis (fig. 26). Normal cartilage cell alignment into long columns, which is so typical of healthy growing bone, was absent.

At the time of sacrifice, 4 phenylalanine deficient rats were placed on a curative diet for 55 days. In all cases the organs and tissues examined, both grossly and histologically, had completely recovered anatomically and functionally (figs. 3, 6, 9, 12, 15, 18, 21, 24, and 27).

DISCUSSION

In common with all work of this type it is difficult to distinguish between the effects, if any, of the lack of the specific dietary element which was omitted from the diet and the effects of the general inanition which results. It seems reasonable to assume, however, that by comparing the results of deficiencies of various essential amino acids it may be possible to infer the roles of the essential amino acids in maintaining the functional state of the various body tissues.

The observations made during the course of this investigation show that the omission of phenylalanine from an otherwise complete diet results in marked changes in the male genital system, in the glomerulosa zone of the adrenal cortex, in the anterior pituitary and in the bones. Comparison of these observations with those reported as resulting from the deficiencies of other amino acids and for starvation, should give some indication as to the specific effects of phenylalanine deficiency.

Siperstein ('20–'21), in his study of the effects of acute and chronic inanition on the testes of the rat, reported changes in the seminiferous tubules with which our results are in agreement. Similar results have been reported by Maun et al. in their work on phenylalanine deficiency ('45a) and leucine deficiency ('45b), and by Adamstone and Spector ('50) for tryptophane deficiency. It thus appears that degeneration of the seminiferous tubules, whether a primary or secondary effect, is common to deficiencies of this type. In regard to the testicular interstitial cells the situation is not so clear. In the study presented here, direct examination of the cells shows degeneration and extreme involution of the prostate and seminal vesicles which implies a decrease in function of the interstitial cells. Previous investigators have not reported effects on the accessory reproductive glands and the results of this investigation are not in complete agreement with their observations on the intersitial tissue. Siperstein ('20–'21), in reporting the effects of inanition, stated that the structure of the interstitial cells was nearly normal except in extreme cases, while Maun and his group reported no significant changes of the interstitial cells resulting from phenylalanine deficiency. The results of this work are in agreement with those of Siperstein in that starved animals on a complete diet showed no interstitial cell degeneration, but reference to figures 7 and 8 will show that in animals deprived of phenylalanine marked changes in the intersitial cells were found. Figure 9 shows recovery in deficient animals placed on a complete diet.

The changes in the adrenal glomerulosa have not been found by others. Maun et al. ('45a) report adrenal cortical changes in the intermediate zone of the cortex which, in the animals examined during this investigation, was unaltered except perhaps in size. Adamstone and Spector ('50) reported no adrenal change resulting from tryptophane deficiency.

Pituitary changes as observed here have not previously been reported. Maun and his collaborators had only a few pituitaries available for study and reported no changes. It is of interest to point out at this time that only in those rats in which atrophy of the adrenal glomerulosa occurred (8 of 10) were the hypophyseal changes previously described noted. In the two animals in which no adrenal cortical alteration was detected, hypophyseal changes were absent.

Several hypothetical sequences of events may be postulated as to the means by which the gonads, adenals and pituitary are involved as a result of phenylalanine deficient diet.

First, the primary effect of the deficiency may be gonadal. This is very unlikely since no castrate cells were observed in the anterior pituitaries and it is well known that surgical or physiological castration results in the appearance of these cells in the anterior pituitary of the rat.

Second, the primary effect of the deficiency may have expressed itself on the adrenal cortex. In an investigation of the effects of adrenal insufficiency on the gonads, Shumaker and Firor ('34) reported that the pituitaries of totally adrenalectomized rats decreased in gonad stimulating power. Gonadal deterioration following total adrenalectomy was also demonstrated by Herrick and Torstveit ('38), Martin and Fazekus ('37) and Freed, Brownfield and Evans ('31). That adrenal insufficiency results in pituitary changes was reported by Martin ('32), Koneff, Holmes and Reese ('41) and Reese, Koneff and Akimoto ('39). These investigators are in agreement that the acidophils regress in size and staining reaction after total adrenalectomy, but not concerning the changes in the basophils, some reporting hyperactivity of these cells, others reporting degeneration. In the present study, no hyperactivity of the basophils could be interpreted from the appearance of these cells. Thus, the possibility of the adrenal glands being the site of primary effect of phenylalanine deficiency is not entirely untenable in view of the gonadal and hypophyseal changes but for the fact that two of the deficient rats had adrenals that were unaltered but in which gonadal deterioration occurred.

Third, the primary effect of the deficiency may have expressed itself on the anterior pituitary so that the secretions of the chromophilic cells could no longer stimulate the adrenals, gonads and general body growth or that the trophic hormones secreted by the pituitary were either incomplete or inadequate. The investigations of Smith ('30), Sarason ('43), and Deane and Greep ('46) indicate that adrenal cortical atrophy results from hypophysectomy. Shumaker and Firor ('34) reported that the dysfunction and atrophy of the reproductive organs of hypophysectomized animals was not a secondary manifestation of the lack of adrenal cortical hormone, since the administration of this hormone failed to repair the reproductive damage and restore sex function. It might be theorized that phenylalanine, among other things, is an essential ingredient for the production of the trophic hormones of the anterior pituitary.

On the basis of the work done thus far the last hypophysis! seems to be the most reasonable, but effects on other tissues and organs independent of any action on the pituitary cannot be excluded. The fact that in 8 of 10 deficient animals pituitary changes were accompanied by adrenal changes, while in two of the 10 animals no changes in either organ could be observed, appears to suggest that the dietary deficiency involved these two tissues primarily. The gonadal changes which are observed in part, at least under conditions of general inanition, might be expected to be aggravated as a result of pituitary deficiency superimposed on a general nutritional imbalance. Hence the assumption that the primary effect of phenylalanine deficiency is on the pituitary is not inconsistent with the results observed. If this is the case, the marked gonadal deterioration observed in the deficient animals as compared with the gradual atrophy resulting from general starvation is not surprising since it may be a secondary effect of pituitary atrophy superimposed on the general inanition.

That the conditions arrived at are completely reversible by dietary change is of extreme interest since it means that in spite of the marked abnormalities found microscopically no permanent damage was done to the normal growth faculties of the tissues involved. It remains yet to be seen, however, whether the reversibility of the structural changes noted in the present work on the return of deficient animals to a complete diet is to be found with deficiencies of other essential amino acids. Investigations with other amino acids are now in progress in this laboratory.

SUMMARY

Male weanling rats on a "synthetic" diet deficient in phenylalanine show marked changes in testes and accessory genital structures, adrenal and pituitary glands, and bone. Pituitary, prostate, epididymal, and adrenal glomerulosal changes have not previously been reported. At the stages of degeneration attained in these experiments the changes are completely reversible with adequate diet. It is suggested that the primary effect of the deficiency is on the pituitary.

LITERATURE CITED

ADAMSTONE, F. B., AND HARRY SPECTOR 1950 Tryptophan deficiency in the rat. Histologic changes induced by the forced feeding of an acid-hydrolyzed casein diet. Arch. Path., *49:* 173.

CANNON, PAUL R. 1948 Some pathological consequences of protein and amino acid deficienices. Charles C. Thomas Publ., Springfield, Illinois.

DEANE, H. W., AND R. O. GREEP 1946 A morphological and histochemical study of the rat's adrenal cortex after hypophysectomy, with comments on the liver. Am. J. Anat., *79:* 117.

FREED, S. C., B. BROWNFIELD AND H. M. EVANS 1931–32 Effect of adrenalectomy on the testes of the rat. Proc. Soc. Exp. Biol. and Med., *29:* 1.

HERRICK, EARL H., AND OLAF TORSTVEIT 1938 Some effects of adrenalectomy in fowls. Endocrin., *22:* 469.

KONEFF, A. A., R. O. HOLMES AND J. D. REESE 1941 Prevention of adrenalectomy changes in the anterior pituitary of the rat by sodium chloride administration. Anat. Rec., *79:* 275.

MARTIN, S. J. 1932 The effect of complete suprarenalectomy on the oestrual cycle of the white rat with reference to suprarenal-pituitary relationship. Am. J. Physiol., *100:* 181.

MARTIN, S. J., AND J. F. FAZEKAS 1937–38 Effect of sodium chloride therapy on oestrous cycle and hypophysis of bilaterally suprarenalectomized rats . Proc. Soc. Exp. Biol. and Med., *37:* 369.

MAUN, M. E., W. M. CAHILL AND R. M. DAVIS 1945a Morphologic studies of rats deprived of essential amino acids. I. Phenylalanine. Arch. Path., *39:* 294.

——— 1945b Morphologic studies of rats deprived of essential amino acids. II. Leucine. Arch. Path., *40:* 173.

REESE, J. D., A. A. KONEFF AND M. B. AKIMOTO 1939 Anaterior pituitary changes following adrenalectomy in the rat. Anat. Rec., *75:* 373.

ROSE, W. C., M. J. OESTERLING AND M. WOMACK 1948 Comparative growth on diets containing ten and nineteen amino acids, with further observations upon the role of glutamic and aspartic acids. J. Biol. Chem., *176:* 753.

SARASON, E. L. 1943 Morphologic changes in the rat's adrenal cortex under various experimental conditions. Arch. Path., *35:* 373.

SHUMACKER, H. B., AND W. M. FIROR 1934 The interrelationship of the adrenal cortex and the anterior lobe of the hypophysis. Endocrin., *18:* 676.

SIPERSTEIN, D. M. 1920–21 Th effects of acute and chronic inanition upon the development and structure of the testis in the albino rat. Anat. Rec., *20:* 355.

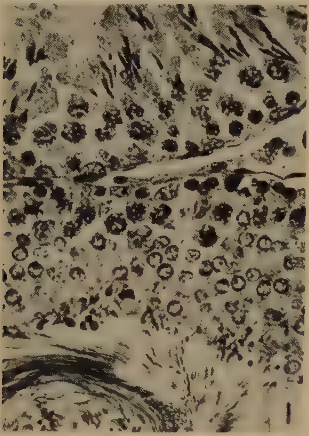
SMITH, P. E. 1930 Hypophysectomy and a replacement therapy in the rat. Am. J. Anat., *45:* 205.

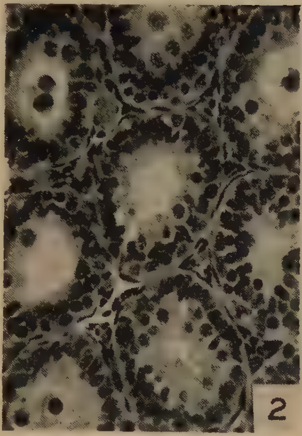
PLATE 1

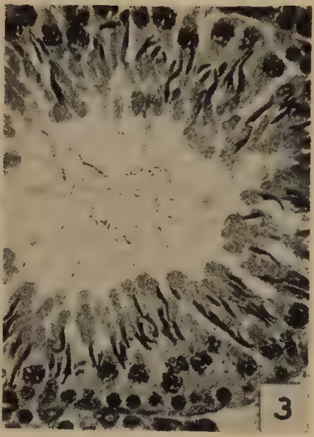
EXPLANATION OF FIGURES

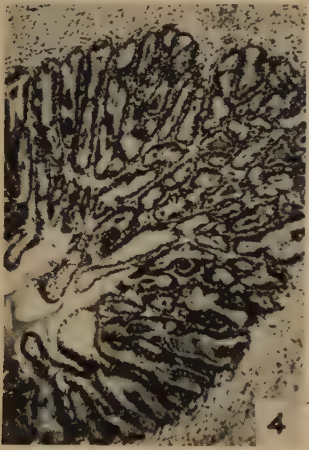
All figures, except figures 22, 23, and 24, are stained with hematoxylin and eosin. Figures 22, 23, and 24 are stained with a modified Mallory's trichrome.

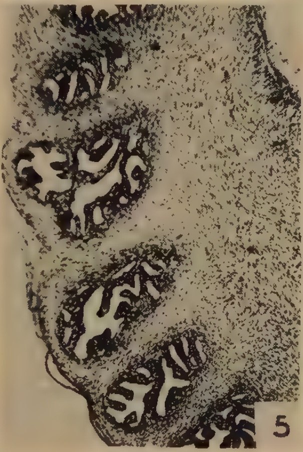
1 Seminiferous tubules of a control rat fed the complete synthetic diet. × 440.
2 Seminiferous tubules of a rat fed the phenylalanine deficient diet. × 440.
3 Seminiferous tubules of a rat placed on a complete recovery diet. × 440.
4 Seminal vesicle of a rat fed the control diet. × 100.
5 Seminal vesicle of a rat deficient in phenylalanine. × 100.
6 Seminal vesicle of a rat fed the complete recovery diet. × 100.
7 Testicular interstitial tissue of a rat on the control diet. × 440.
8 Testicular interstitial tissue of a phenylalanine deficient rat. × 440.
9 Testicular interstitial tissue of a rat fed the complete recovery diet. × 440.

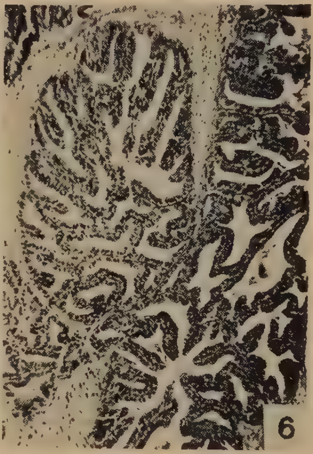

1

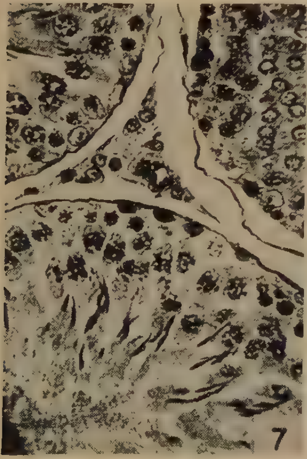

2

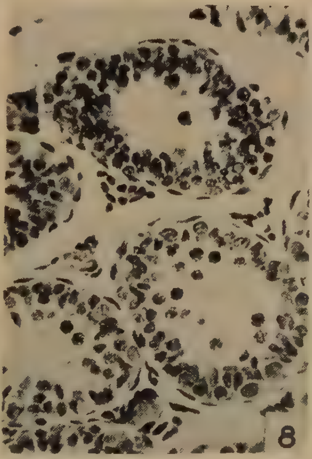

3


4


5


6


7


8

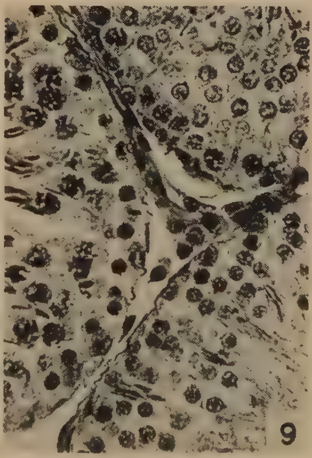
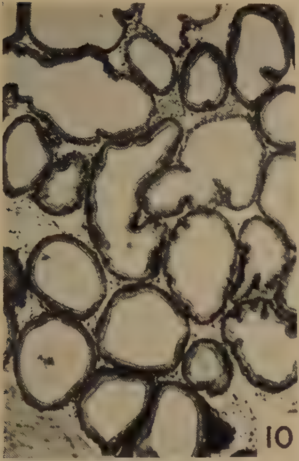
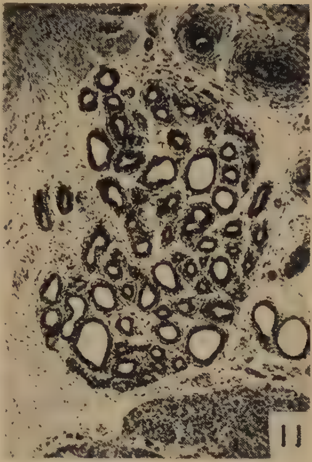

9

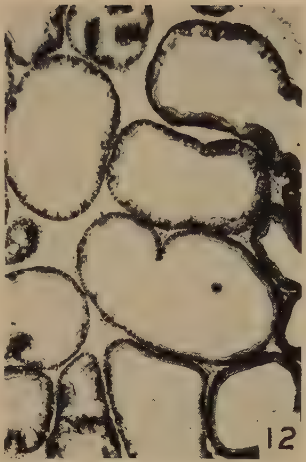
PLATE 2

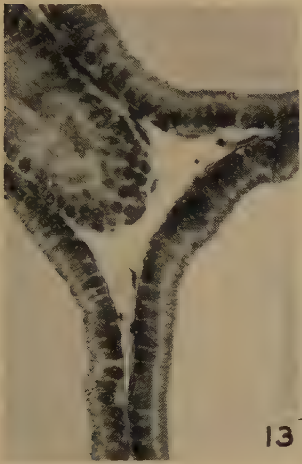
EXPLANATION OF FIGURES

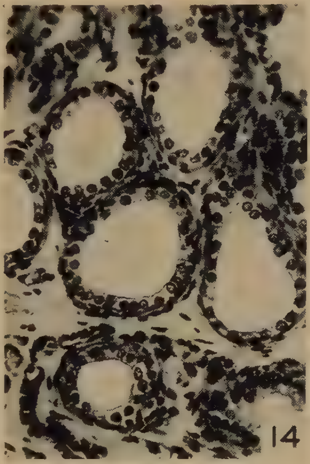
10 Ventral prostate of a rat on the control diet. × 100.
11 Ventral prostate of a rat fed the phenylalanine deficient diet. × 100.
12 Ventral prostate of a rat fed the complete recovery diet. × 100.
13 Prostatic epithelium of a rat on the control diet. × 440.
14 Prostatic epithelium of a rat fed the phenylalanine deficient diet. × 440.
15 Prostatic epithelium of a rat fed the complete recovery diet. × 440.
16 Adrenal cortex of a rat on the control diet. × 100.
17 Adrenal cortex of a rat fed the phenylalanine deficient diet. × 100.
18 Adrenal cortex of a rat fed the complete recovery diet. × 100.

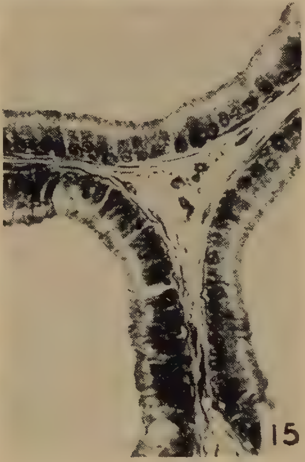

10

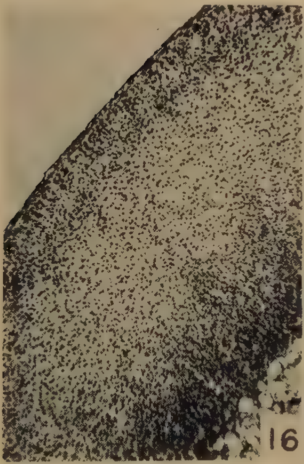

11

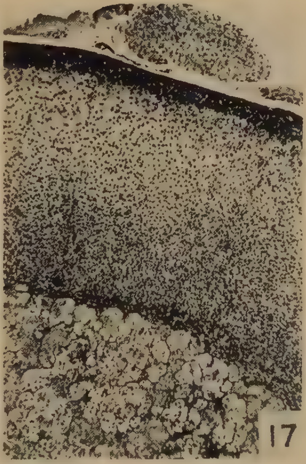

12


13


14


15


16


17

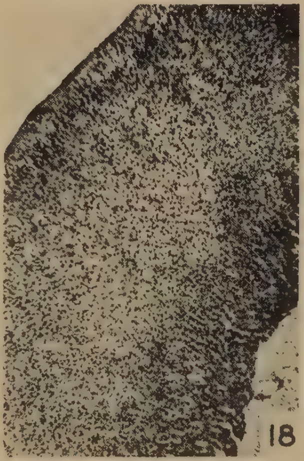

18

PLATE 3

EXPLANATION OF FIGURES

19 Thyroid gland of a rat on the control diet. × 100.

20 Thyroid gland of a rat on the phenylalanine deficient diet. × 100.

21 Thyroid gland of a rat fed the complete recovery diet. × 100.

22 Acidophils of the anterior pituitary of a control rat. × 900.

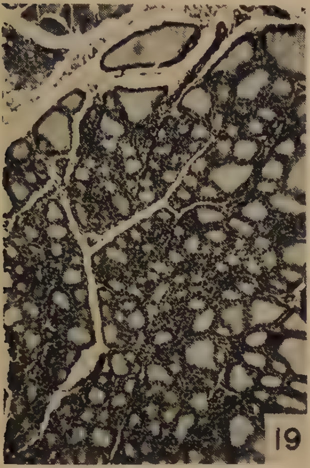
23 Acidophils of the anterior pituitary of a rat on the phenylalanine deficient diet. × 900.

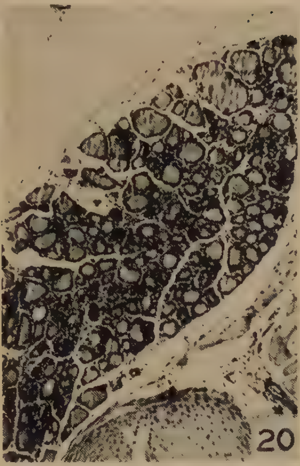
24 Acidophils of the anterior pituitary of a rat on the complete recovery diet. × 900.

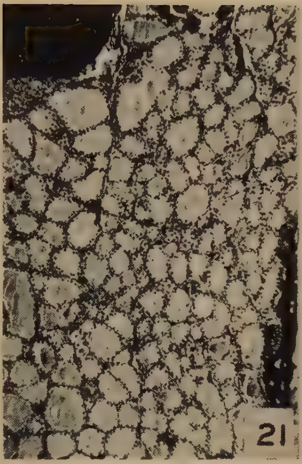
25 Epiphyseal plate of the tibia of a control rat. × 100.

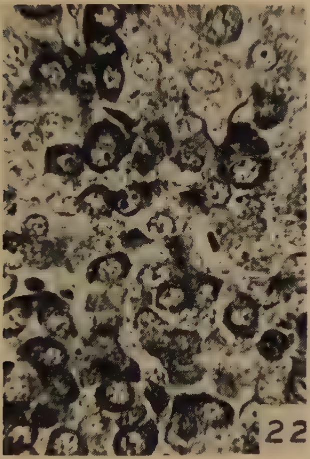
26 Epiphyseal plate of the tibia of a rat fed the phenylalanine deficient diet. × 100.

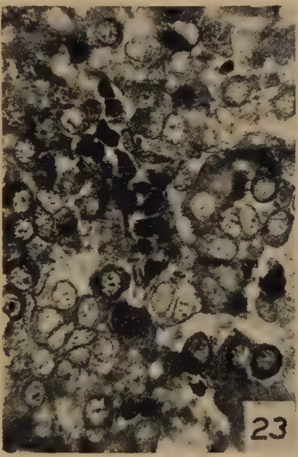
27 Epiphyseal plate of the tibia of a rat placed on the complete rcovery diet. × 100.

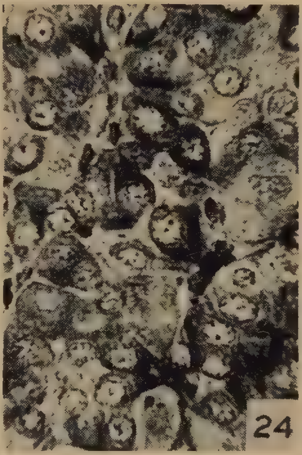

19

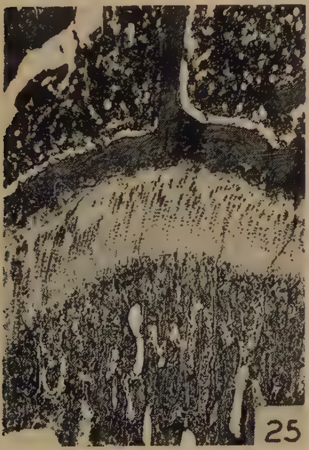

20

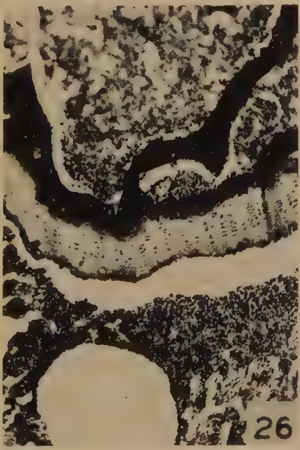

21

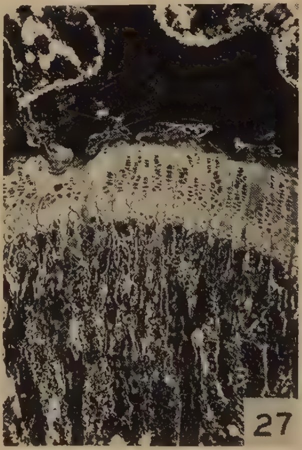

22


23


24


25


26


27

OBSERVATIONS ON THE FORMATION OF THE SECOND POLAR BODY IN THE RAT OVUM[1]

D. LOUISE ODOR
Department of Anatomy, University of Washington School of Medicine, Seattle
AND
RICHARD J. BLANDAU
Department of Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, New York

SIXTEEN FIGURES

INTRODUCTION

There are many details in the formation of the second polar body in mammalian ova which have been either incompletely described or which are entirely lacking. By utilizing phase microscopy, a number of interesting features of second polar body formation have been observed in living rat ova and these observations have been compared with the details of structure noted in fixed and stained preparations. The fresh rat ovum is particularly well suited to a study of this type since the formed constituents of its cytoplasm are neither as large nor as numerous as those of many other mammalian ova.

The manner in which the second polar body is formed in mammalian eggs appears to be quite similar in those animals in which this phenomenon has been studied adequately. With the possible exception of the mouse (Lams and Doorme, '08; Gresson, '48) and the dog (O. Van der Stricht, '23), the freshly ovulated mammalian ova (Sobotta and Burckhard,

---

[1] This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council, and the Dr. Henry C. Buswell Memorial, University of Rochester, School of Medicine and Dentistry, Rochester, New York.

'10; Kirkham and Burr, '13; Rubaschkin, '05; Lams, '13; Pincus and Enzmann, '32; O. Van der Stricht, '23; Pearson and Enders, '43; Hamilton, '44; Wright, '48) are in the metaphase stage of the second maturation division at the time of sperm penetration. The cytological changes occurring during the formation of the second polar body have been described in considerable detail for the ova of relatively few mammals, namely, the white mouse (Lams and Doorme, '08), the guinea pig (Rubaschkin, '05; Lams, '13), the rabbit (Van Beneden, 1875), the cat (R. Van der Stricht, '11) and the bat (O. Van der Stricht, '10). Similar studies have been reported on many of the lower vertebrates and invertebrates, for example Amphioxus (O. Van der Stricht, 1896), Axolotl (Jenkinson, '04), Ciona (Duesberg, '15), Ascaris (Van Beneden, 1883), Thalassema and Zirphaea (Griffin, 1899), Chaetopterus (Mead, 1898), and Limax (Byrnes, 1899).

There is no adequate information for any mammal regarding the temporal relationship between the entrance of the fertilizing sperm, its transformation into the male pronucleus and the abstrusion of the second polar body. Pincus and Enzmann ('32) reported that in rabbit ova 45 minutes or more elapse from the time of sperm entry until the second polar body is completely formed. Considerably more data regarding this time interval have been compiled for some of the lower vertebrates (Triturus [Fankhauser and Moore, '41]; Diemyctylus [Jordan, 1893]; Cryptobranchus [Smith, '12]; Bufo [King, '01]). Specifically for the rat, Coe ('08) and Kirkham and Burr ('13) state that the second polar body is extruded immediately after sperm penetration. Sobotta and Burckhard ('10), on the other hand, express the opinion that a considerable elapse of time is required for the completion of the second polar body in this animal.

This paper presents information concerning: (1) the cytological changes occurring in the rat ovum during the formation of the second polar body, and (2) the temporal relationship of the entrance of the fertilizing sperm and its subsequent transformation into the male pronucleus with the changes leading to the abstriction of the second polar body.

MATERIALS AND METHODS

Sexually mature, female, albino rats of the Wistar and Vanderbilt strains were used in this study. The onset of behavioral estrus is an accurate reference point in the estrous cycle of the rat and was used in this study for estimating the approximate time of ovulation. A female rat at the onset of, and during heat responds to manual manipulation of the pudendal region with a typical lordosis which is accompanied by a definite ear quiver (Blandau, Boling and Young, '41). After the onset of heat was determined, each female was placed with two vigorous males and allowed to mate overnight. If, on the following morning, a vaginal smear showed the presence of sperm, the animal was killed by decapitation at the desired interval after ovulation.

Since polar body formation and subsequent pronuclear changes occur within the region of the dilated portion of the ampulla of the oviduct, only this portion need be removed to obtain ova for examination. If the ova were to be sectioned, the whole dilated loop of the ampulla was placed in Zenker-formol solution. The tissues were dehydrated in graded ethyl alcohol, embedded in paraffin (56–58°C.) and sectioned serially at 6 μ. Most of the sectioned material was stained with Heidenhain's iron hematoxylin, the remainder with Giemsa stain.

The procedure for observing the living ova may be outlined as follows: Immediately after decapitation, the ovaries with their attached oviducts, were removed from the animal. The ampullae were dissected from the ovaries under a binocular dissecting microscope. A fine glass pipette filled with Ringer-Locke's solution was inserted into the fimbriated end of each oviduct. The fluid was then forced through the ampulla under slight pressure, thereby flushing out the ova. Any granulosa cells surrounding the ova were removed by the addition of small quantities of the enzyme hyaluronidase.[2] Each ovum was separately taken up into a capillary pipette in a small amount of Ringer-Locke's solution. It was transferred onto a glass slide and was surrounded with a thin vaseline ring of about 1 cm in diameter. The vaseline ring was prepared in such a manner so as to leave a 2–3 mm gap at one side. An 18 mm round cover glass was lowered carefully onto the vaseline ring. After the cover glass was sealed in position a small drop of mineral oil was placed at the edge of the cover glass adjacent to the opening in the vaseline ring to completely seal the preparation. This method of preparation allows for the more gentle flattening of the specimen as the observations proceed in that the enclosed air or fluid may readily escape via the gap by pushing through the more liquid mineral oil. All of the living ova were observed with the phase microscope employing the dark contrast medium objectives. As each ovum was examined the spatial relationships of intraovular structures were first determined, the position of the polar body serving as a landmark. The ovum was gradually flattened by gently applying pressure to the cover glass with a fine, pointed, dental probe. By observing the egg directly as the pressure was applied the degree of flattening could be controlled and any structural displacements noted. Observations were described in detail and recorded photographically. Photographs were taken on either Contrast Process Panchromatic or DuPont High Speed Pan Type, no. 428 film.

The observations to be described in this paper are based on the examination of 1,934 rat ova removed from 268 animals. Of these 1,304 were observed in the living state and 630 were examined after histologic preparation.

OBSERVATIONS AND DISCUSSION

As described earlier (Blandau, '45) the first polar body of the rat ovum begins to be formed during the period of preovulatory follicular swelling and is completely abstrused at the time of ovulation.

---

[2] The enzyme hyaluronidase was supplied by the Ortho Research Foundation, Raritan, New Jersey, through the courtesy of Dr. Carl G. Hartman.

The genesis of the second maturation spindle was not determined in the present work since the material was collected primarily for the study of fertilization phenomena. However, the metaphase stage of the second maturation division was observed in numerous unfertilized living and sectioned tubal ova. In sectioned ova, such as that shown in figure 1, the spindle of the second maturation division is observed to be elongated and quite narrow. It is invariably located paratangentially to the surface of the egg. The intensely staining chromosomes are closely aggregated at the equator of the spindle in what appears to be a flattened plate. In ova stained with either Giemsa or iron hematoxylin in which the destaining process has been allowed to continue until the oöplasm loses much of its color, the metaphase plate has an irregular contour in which small groups of chromosomes appear to project beyond the dense central area. This point is illustrated in figures 2 and 3 which are photographs of the chromosomal plate of two different metaphase spindles as seen in cross and tangential section respectively. The entire metaphase plate of the spindles in these figures was contained in a single section 6 μ in thickness. The spindle fibers are numerous and well delineated in iron hematoxylin preparations. They appear as very fine filaments which stain more darkly than the suurounding oöplasm. In Giemsa stained ova the fibers appear slightly more basophilic than the oöplasm. No distinct centrioles could be detected at the poles of the metaphase spindles but usually several darkly stained granules, somewhat larger than the general oöplasm granules, were visible in the region of the poles. Their significance is as yet unknown. Sobotta and Burckhard's ('10) description of the metaphase of the second maturation division in the rat ovum is in general agreement with that presented above. There is a minor point of difference in that they describe and figure the shape of the chromosomes as being round, rather than rod-like. They suggest that the small darkly stained granules at the poles of the spindle are mitochondria. Coe ('08) states that the metaphase spindle of the rat ovum ". . . contains either dyads or the separated and often rod-like univalent chromosomes." Our observations on the contour of the chromosomes are in agreement with those of Coe.

Only occasionally could the metaphase spindle as a whole be visualized in living ova. When seen it appeared as a barrel-shaped, non-granular body with a compact mass of chromosomes located in the equatorial plane. In such spindles neither spindle fibers nor centrioles were observed. By appropriate manipulation it was possible to disengage the intact metaphase plate of chromosomes from the öoplasm in a number of fresh ova. The chromosomes appeared to be embedded in a relatively tenacious, homogeneous, non-granular bit of cytoplasm. On tapping the cover glass the metaphase plate could be moved about in the Ringer-Locke's solution as a unit and appeared as a biconvex disc with irregular edges. By carefully flattening the isolated metaphase plate the individual chromosomes were more readily visualized (figs. 4 and 5). They appeared as short, straight or slightly curved rods with rounded ends. The chromosomes in this stage were uniformly of high contrast. In several preparations one portion of the freed metaphase plate became adherent to the glass slide and secondarily to the cover glass. By gently moving the cover glass the chromosomes could be stretched into long, exceedingly thin fibers without being broken. The stretched fibers did not appear homogeneous but showed segmentally arranged areas of higher contrast. When the cover glass was returned to its original position, thereby relieving tension on the stretched chromosomes, they returned to their normal configuration. The metaphase chromosomes as seen in fresh preparations have not been previously described for mammalian ova.

Unless fertiliziation occurs no further progress is made toward the completion of the second polar body. Extensive unpublished data on the relationship of ovulation to the time of fertilization in mated rats has shown that 90% of the ova are fertilized within three hours after ovulation, while only 47% of the ova examined during the first hour after ovulation were fertilized. It is important to note that within a group of eggs in a single oviduct there may be a variation of from one to three hours in fertilization time. Because of the variability in the time required for the sperm to reach the oöplasm, not all of the ova are in the same stage of polar body formation if examined between the time interval mentioned.

A number of sectioned and living ova were observed in which the sperm had apparently entered the oöplasm just before either fixation or observation. The chromosomes of the second maturation division were aggregated on the metaphase plate although an unchanged sperm head was present within the peripheral part of the oöplasm. This stage is probably of short duration for it was rather infreqeuntly encountered in this study and has not been described for the rat by other investigators. Such a stage, however, was pictured in a sectioned guinea pig ovum by Lams ('13).

Anaphase stages, not previously reported in the literature, were very infrequently encountered and then were seen only in sectioned ova. An early anaphase spindle is shown in figure 6. The chromosomes lie a short distance from the equator and are not as closely aggregated as those on the metaphate plate (compare with fig. 1). Even though the spindle fibers are distinctly visible, the mid-body of the spindle is not seen in this stage. The head of the fertilizing sperm which lies at the opposite pole in this ovum has undergone no visible change. Further progress in the migration of the chromosomes is shown in figure 7. Localized thickenings of the spindle fibers in the region of the equator may now be visualized. The head of the sperm in this specimen had not begun to transform into the male pronucleus.

Early telophase stages have been encountered much more frequently in both living and sectioned ova. Figure 8 a illustrates a telophase spindle as observed in the oöplasm of a living, fertilized eg. The apices of the spindle are broad and plate-like and there are well defined spindle fibers enclosed in a non-granular protoplasm. On rotation of the living egg the whole spindle appeared as a cigar-shaped cylinder. Figure 8 b is a photograph of the same spindle shown in figure 8 a but in this case only the most superficial part of the spindle was brought into focus. Elongated, localized thickenings, of higher contrast than the fibers themselves, may be seen on each fiber at the equator of the spindle. These thickenings, observed as a group, may be defined as the mid-body. In sectioned ova the region of the mid-body stains intensely both with iron hematoxylin and Giemsa stains.

A somewhat more advanced telophase spindle of a living ovum is shown in figure 9. In this egg the polar body had not been abstricted. The chromosomes at each pole of the spindle have become more closely grouped into single masses with irregular contours. The mid-body and spindle fibers are visible. For comparison, figure 10 is a photograph of a sectioned ovum in which polar body formation has advanced to approximately the same stage as that shown in figure 9. The chromosomes are aggregated into an even more compact body, although the ends of several chromosomes may be visualized protruding from the main mass. The mid-body is seen at the equator of the spindle. Comparable early telophase spindles have been described by Sobotta and Burckhard ('10) in the sectioned rat ovum and by O. Van der Stricht ('23) in the sectioned bat egg.

The *in situ* appearance of a telophase spindle of a living ovum at a slightly later stage in development than that shown in figure 9 may be seen in figure 11. The chromosomal masses at the poles are iregularly shaped but appear quite homogeneous. The mid-body may be readily seen, but the spindle fibers are not clearly visible.

The head of the fertilizing sperm begins its transformation into the male pronucleus at the time when the early telophase stage of the second polar body formation has reached a point in its development comparable to that shown in figure 11. The first observable alteration in the head of the sperm is the enlargement of its most posterior region. This enlargement is accompanied by a loss of contrast of this region in the fresh specimen or a decrease in stainability in the fixed preparation. A correlation of the initial transformation of the sperm head with any specific stage in second polar body formation has not been reported previously for any mammal.

The final stage in the formation of the polar body is its complete extrusion and separation from the oöplasm (figs. 12, 13, 14). The chromosomes in both the polar body and oöplasm at this stage have formed dense, homogeneous appearing bodies with little visible structural detail. The chromosomal mass within the polar body usually assumes an elongated, irregular form (figs. 12 and 14). The chromosomal mass within the ovum ordinarily is ovoid in shape and compacted. In living ova both of the chromosomal masses have a high contrast. In sectioned ova the abstrused polar body usually contains a number of small, darkly stained, spheroid or ovoid bodies in addition to the chromosomal mass. These bodies are larger than oöplasmic granules and are less basophilic in Giemsa preparations than the chromosomal mass. Their significance is as yet unknown.

Even though the second polar body forms a separate ovoid vesicle at this stage, it may remain adherent to the oöplasm by the spindle fibers. In such living ova (fig. 15) the midbody appears as a small ring-shaped structure in which spindle fibers are projected. By tapping on the cover glass before detachment of the spindle fibers from the oöplasm the polar body may be moved about vigorously without becoming loosened from the ovum, emphasizing the fact that the spindle fibers have a certain amount of resiliency and strength.

As shown in figure 16 the polar body may be manipulated in such a manner that its aggregated group of spindle fibers may be pulled out intact from the oöplasm. In such cases in which the spindle is removed intact the fibers composing it may be compacted together and the whole spindle may be moved about as a unit. In several instances the spindle fibers became separated into a fan-shaped arrangement in which the individual fibers could be observed. On being moved about they were bent easily but upon release of pressure invariably returned to their original shape and position.

The stage of maturation in which the second polar body has been completely extruded from the ovum was most frequently observed both in this study and in the reports of other investigators (rat [Sobotta and Burckhard, '10; Kremer, '24; Kirkham and Burr, '13]; white mouse [Lams and Doorme, '08]; dog [O. Van der Stricht, '23]; bat [O. Van der Stricht, '10]; and guinea pig [Lams, '13]).

In the majority of rat ova the second polar body had been abstricted by the end of the 4th hour after fertilization. By this time the entire head of the fertilizing sperm had become greatly enlarged. The stages in the formation of both the male and female pronuclei and the fate of the sperm middle-piece and tail will be described in detail in subsequent papers.

SUMMARY

1. Utilizing the phase microscope metaphase and telophase stages of the second maturation division have been observed in living rat ova. Metaphase, anaphase and telophase stages were also studied in sectioned ova.

2. It was possible to disengage the chromosomes from the oöplasm of living ova in the metaphase stage and thus to gain added information regarding their morphological features.

3. Two phases of the migration of the chromosomes from the equator to the poles of the spindle were observed. The mid-body first became visible during the late anaphase stage.

4. There is no detectable change in the fertilizing sperm head until the early telophase stage of the second maturation division is reached. It is during this interval that the posterior region of the head of the sperm begins to enlarge. The mid-body was more easily visualized at this time. The spindle fibers are plainly visible in living ova in both early and late telophase stages.

5. During the late telophase stage the second polar body is abstrused from the oöplasm. In the living egg the midbody assumes the appearance of a ring through which the spindle fibers pass from the chromatin mass in the polar body to the primordium of the female pronucleus in the oöplasm. At this time the sperm head has become uniformly enlarged and is beginning to lose its high contrast.

LITERATURE CITED

Blandau, R. J. 1945 The first maturation division of the rat ovum. Anat. Rec., *92:* 449–457.

Blandau, R. J., J. L. Boling and W. C. Young 1941 The length of heat in the albino rat as determined by the copulatory response. Anat. Rec., *79:* 453–463.

Byrnes, E. F. 1899 The maturation and fertilization of the egg of Limax agrestis (Linné). J. Morph., *16:* 201–236.

Coe, W. R. 1908 The maturation of the egg of the rat. Science, N. S., *27:* 444–445.

Duesberg, J. 1915 Recherches cytologiques sur la fécondation des Ascidiens et sur leur développement. Carnegie Contrib. Embryol., *3:* 33–70.

Fankhauser, G., and C. Moore 1941 Cytological and experimental studies of polyspermy in the newt, Triturus viridescens. I. Normal fertilization. J. Morph., *68:* 347–385.

Gresson, R. A. R. 1948 Fertilization, parthenogenesis, and the origin of the primitive germ-cells of some animals. Ch. VIII, 64–75. Essentials of General Cytology, Edinburgh at the University Press.

Griffin, B. B. 1899 Studies on the maturation, fertilization, and cleavage of Thalassema and Zirphaea. J. Morph., *15:* 583–634.

Hamilton, W. J. 1944 Phases of maturation and fertilization in human ova. J. Anat., *78:* 1–4.

Jenkinson, J. W. 1904 Observations on the maturation and fertilization of the egg of the Axolotl. Quar. J. Micr. Sci., N. S., *48:* 407–482.

Jordan, E. O. 1893 The habits and development of the newt (Diemyctylus viridescens). J. Morph., *8:* 269–366.

King, H. D. 1901 The maturation and fertilization of the egg of Bufo lentiginosus. J. Morph., *17:* 293–350.

Kirkham, W. B., and H. S. Burr 1913 The breeding habits, maturation of eggs and ovulation of the albino rat. Am. J. Anat., *15:* 291–317.

Kremer, J. 1924 Das Verhalten der Vorkerne im befruchteten Ei der Ratte und der Maus mit besonderer Berücksichtigung ihrer Nucleolen. Ztschr. f. mikr.-anat. Forschung, *1:* 353–390.

Lams, H. 1913 Etude de l'oeuf de Cobaye aux premiers stades de l'embryogenèse. Arch. de Biol., *28:* 229–323.

Lams, H., and J. Doorme 1908 Nouvelles recherches sur la maturation et la fécondation de l'oeuf des mammifères. Arch. de Biol., *23:* 259–365.

MEAD, A. D. 1898 The origin and behavior of the centrosomes in the annelid egg. J. Morph., *14:* 181–218.

PEARSON, O. P., AND R. K. ENDERS 1943 Ovulation, maturation and fertilization in the fox. Anat. Rec., *85:* 69–83.

PINCUS, G., AND E. V. ENZMANN 1932 Fertilization in the rabbit. J. Exp. Biol., *9:* 403–408.

RUBASCHKIN, W. 1905 Über die Reifungs- und Befruchtungsprozesse des Meerschweincheneies. Anat. Hefte, *29:* 509–553.

SMITH, B. G. 1912 The embryology of Cryptobranchus allegheniensis. including comparisons with some other vertebrates. I. Introduction; the history of the egg before cleavage. J. Morph., *23:* 61–157.

SOBOTTA, J., AND G. BURCKHARD 1910 Reifung und Befruchtung des Eies der weissen Ratte. Anat. Hefte, *42:* 433–497.

VAN BENEDEN, E. 1875 La maturation de l'oeuf, la fécondation, et les premières phases du développement embryonnaire des mammifères d'après des recherches faites chez le Lapin. Bull. Acad. roy. de Belgique, 2me Ser., *40:* 686–736.

——— 1883 Recherches sur la maturation de l'oeuf et la fécondation. Ascaris megalocephala. Arch. de Biol., *4:* 265–640.

VAN DER STRICHT, O. 1896 La maturation et le fécondation de l'oeuf d'Amphioxus lanceolatus. Arch. de Biol., *14:* 469–495.

——— 1910 La structure de l'oeuf mammifères (Chauve-souris, Vesperugo noctula). Troisième partie. L'oocyte à la fin du stade d'accroissement, au stade de la maturation, au stade de la fécondation et au début de la segmentation. Acad. roy. de Belgique, Classe des Sciences, Memoires, 2me Ser., *2:* 1–176.

——— 1923 Etude comparée des ovules des mammifères aux différentes périodes de l'ovogenèse, d'après les travaux du Laboratoire d'Histologie et d'Embryologie de l'Université de Gand. Arch. de Biol., *33:* 229–300.

VAN DER STRICHT, R. 1911 Vitellogenèse dans l'ovule de Chatte. Arch. de Biol., *26:* 365–481.

WRIGHT, P. L. 1948 Preimplantation stages in the long-tailed weasel (Mustela frenata). Anat. Rec., *100:* 593–607.

PLATES

PLATE 1

EXPLANATION OF FIGURES

All photomicrographs of the fixed ova on this plate were made from sections 6 μ in thickness, stained with iron hematoxylin. The photomicrographs of the living specimens are from eggs examined with the dark contrast medium objectives of the phase microscope. ♂ = sperm head.

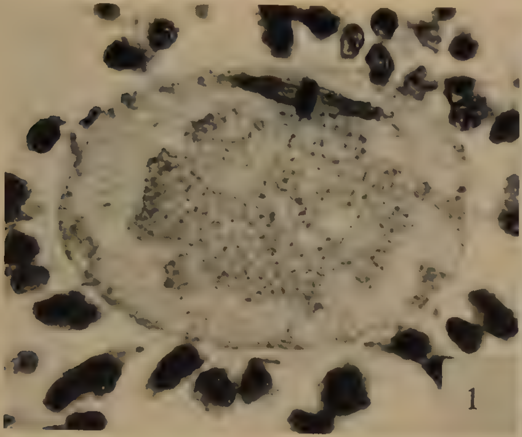
1 Unfertilized, sectioned tubal egg showing the entire metaphase spindle of the second maturation division as seen in a longitudinal view. × 900.

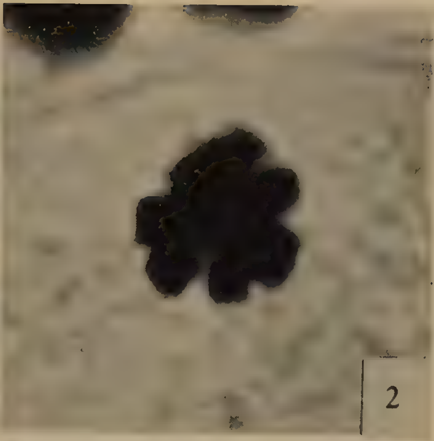
2 Cross section of metaphase chromosomal plate of second maturation spindle of an unfertilized ovum. × 5400.

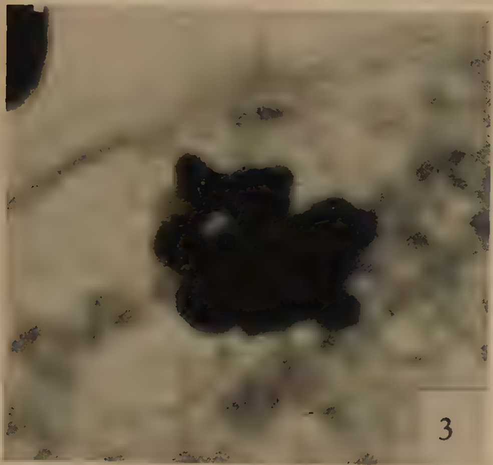
3 Tangential section through a metaphase chromosomal plate of second maturation spindle of an unfertilized egg. × 5400.

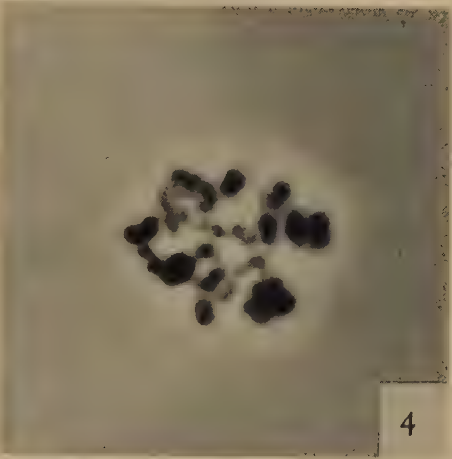
4 and 5 Appearance of chromosomes on a metaphase plate of second maturation spindle. Each aggregate of chromosomes was isolated from a living unfertilized ovum. Note the clear cytoplasm in which the chromosomes are imbedded. × 2580.

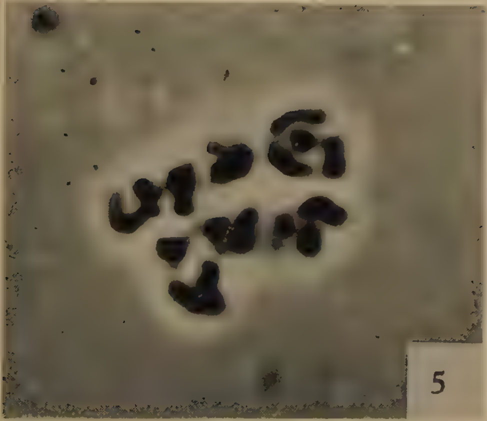
6 Early anaphase spindle of a sectioned, fertilized ovum. The unaltered head of the fertilizing sperm is near the right border of the egg. × 900.


1


2


3


4


5

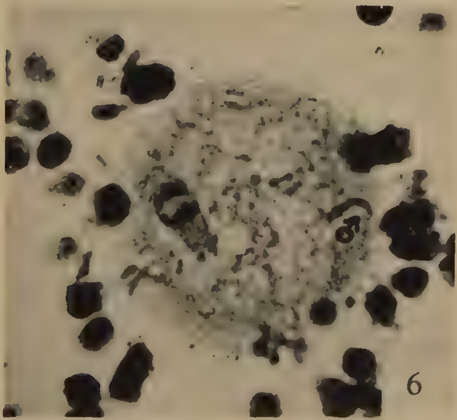

6

PLATE 2

EXPLANATION OF FIGURES

All photomicrographs of the fixed ova on this plate were made from sections 6 μ in thickness, stained with iron hematoxylin. The photomicrographs of the living specimens are from eggs examined with the dark contrast medium objectives of the phase microscope. ♂ = sperm head, ♀ = primordium of female pronucleus, A = mid-body, C = chromosomal mass.

7 Late anaphase spindle of sectioned, fertilized egg. The head of the fertilizing sperm is seen near the lower border of the ovum. It had undergone no detectable transformation. × 900.

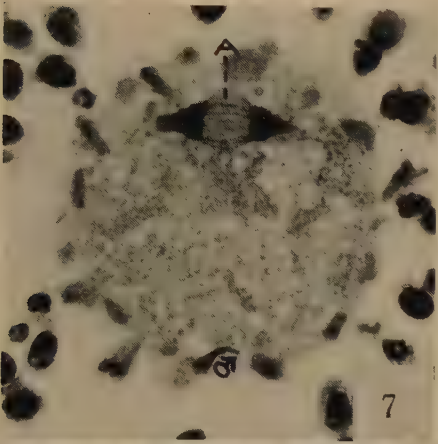
8 a and 8 b Early telophase spindle of second maturation division as observed in the oöplasm of a living, fertilized ovum. In 8 a it may be seen that the two chromosomal groups have not as yet become compact, homogeneous masses. Note the increase in contrast of the individual spindle fibers in the region of the mid-body in 8 b. × 1746.

9 Early telophase spindle of a living, fertilized egg. This is a slightly more advanced stage than that seen in figure 8. The groups of chromosomes are more compact. The spindle fibers and mid-body may be noted. × 1746.

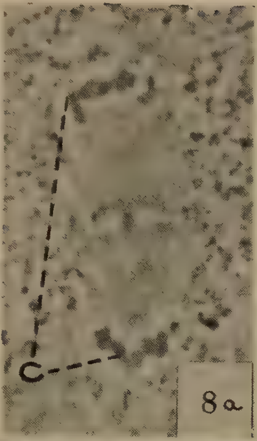
10 Early telophase spindle of a sectioned, fertilized egg. The polar body had not been abstricted. × 900.

11 A somewhat more advanced telophase spindle of a living, fertilized ovum. The head of the fertilizing sperm in this egg was somewhat enlarged. × 2580.

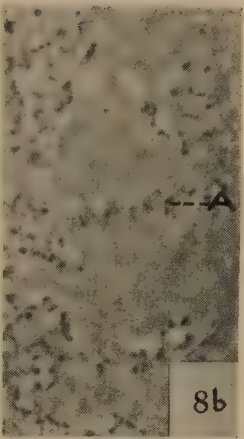
12 Late telophase spindle of a living, fertilized ovum. The second polar body had been extruded from the ovum. The chromosomal masses in both the polar body and oöplasm are more compact and the female pronucleus is assuming a more round appearance. The fertilizing sperm head had become completely enlarged. × 1935.



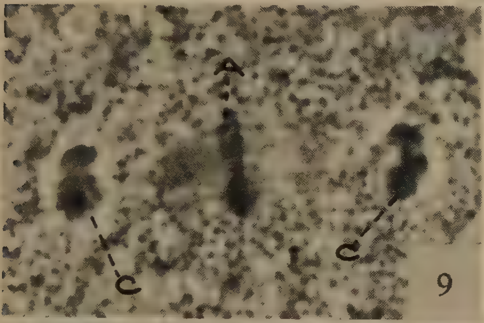
7



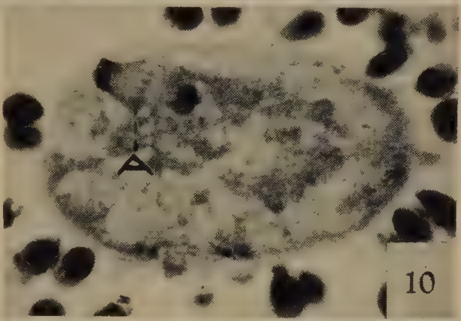
8a



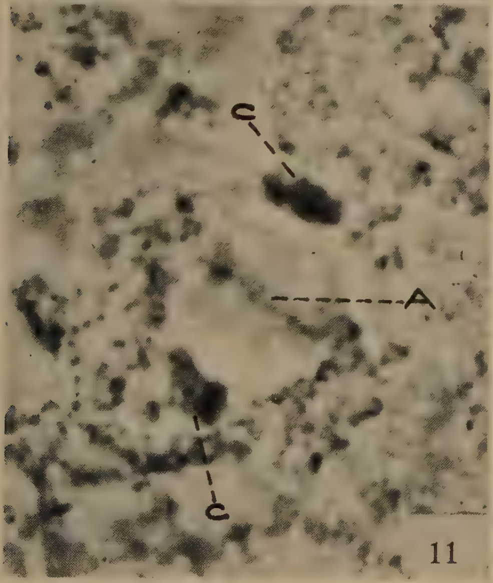
8b



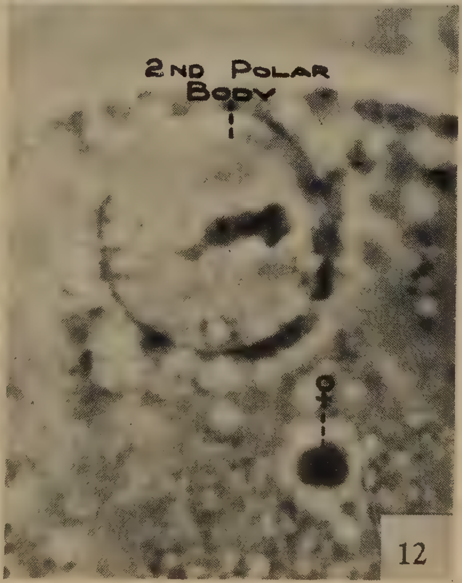
9



10



11



12

PLATE 3

EXPLANATION OF FIGURES

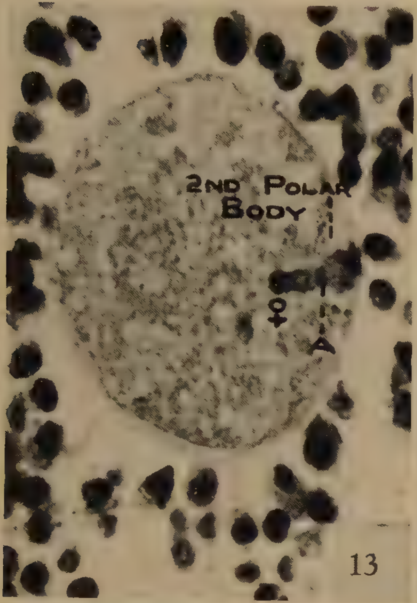
The photomicrograph of the fixed ovum on this plate was made from a section 6 μ in thickness, stained with iron hematoxylin. The photomicrographs of the living specimens are from eggs examined with the dark contrast medium objectives of the phase microscope. ♂ = sperm head, ♀ = primordium of female pronucleus, A = mid-body, B = perforatorium of sperm head.

13 Late telophase spindle of a sectioned, fertilized egg. The second polar body had been constricted from the ovum. The primordium of the female pronucleus is present in the oöplasm as a dense mass still attached to the polar body by spindle fibers. A prominent mid-body is visible. × 900.

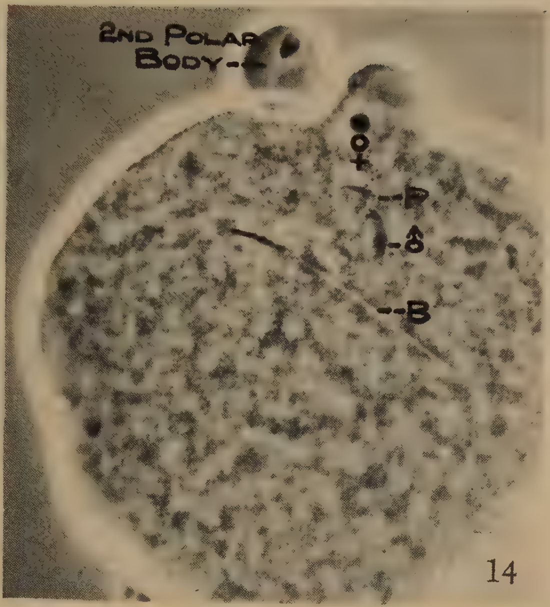
14 Living, fertilized egg. The polar body had been abstrused. The chromosomal mass in the polar body and the primordium of the female pronucleus have a high contrast. The sperm head is condensing to form the male pronucleus. The perforatorium of the sperm head and the sperm middle-piece are visible in the oöplasm. × 645.

15 Second polar body of living fertilized ovum. The mid-body has the appearance of a ring through which the spindle fibers project from the polar body toward the oöplasm. The second polar has relatively few cytoplasmic inclusions and organelles. × 1455.

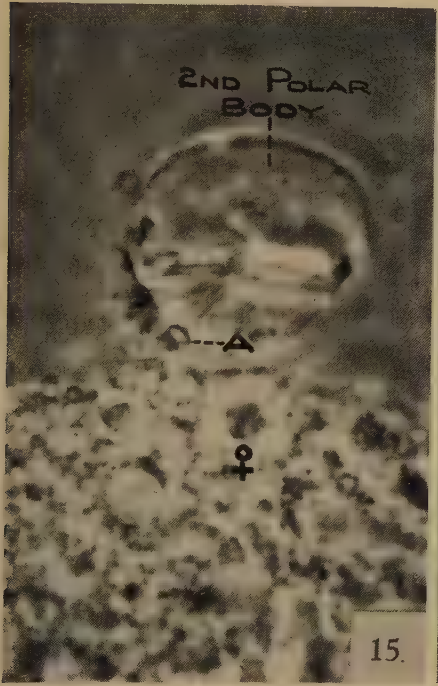
16 Second polar body which had been pulled away from the oöplasm in a living ovum. The nuclear mass within the polar body is still attached to spindle fibers. Note that the spindle fibers extending beyond the polar body are still present as distinct units. × 1746.


2ND POLAR BODY
♀
A

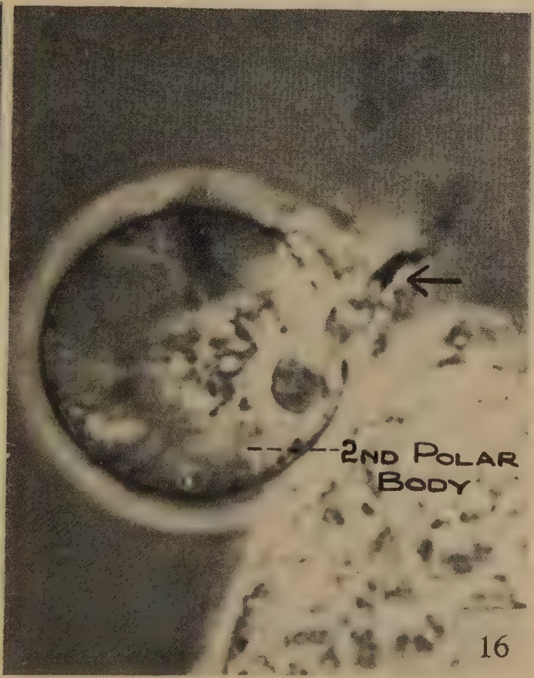
13


2ND POLAR BODY
♀
P
♂
B

14


2ND POLAR BODY
A
♀

15.


2ND POLAR BODY

16

THE WEIGHT OF THE EYEBALLS IN THE GUINEA PIG

HOMER B. LATIMER
Department of Anatomy, University of Kansas

Most of the weights of the eyeballs given in the literature are for only a few specimens and not enough for statistical treatment. In many cases the weights in grams are given without the total body weight, so that the relative weights cannot be compared.

The growth of the eyeballs in the guinea pig has been studied by Bessesen and Carlson ('23) and the weights of the eyeballs from 12 male guinea pigs are given by Crile and Quiring ('40). The growth of the eyeballs in the albino rat is given by Jackson ('13), and the growth of the human eye, including some data on the adult eye, has been reported by Scammon and Armstrong ('25). Latimer ('25, '27, '38) has given the weights of the eyeballs in the chicken, turkey and the adult cat. Much of the literature on the weights of the eyeballs is reviewed by Oppenheim ('47) and by Steindorf ('47). Crile and Quiring ('40) give the weights of the eyeballs for many animals of widely different species, but in most cases there are only a few specimens of each species.

This series of guinea pig eyes is larger than any series of adult eyes of a single species found so far in the literature, and it is hoped that it will contribute not only to our knowledge of the variability in weights and percentage weights, but as well give some idea of the correlations with some of the other organs.

MATERIALS AND METHODS

The 100 young adult male guinea pigs used in this study were the same as those whose brain and cord weights have been reported (Latimer, '50), and the details of body weight, body length and methods of dissection need not be repeated. The two eyeballs were carefully removed and all fat, muscles and nerves carefully severed close to the eyeball leaving the eyeball as clean as possible. The two eyeballs were placed in a moist chamber until weighed in a glass stoppered weighing bottle on an analytical balance sensitive to 0.1 mg.

The usual statistical constants were determined and the regression lines were determined by the method of least squares (Snedecor, '38) . The weights of the two eyeballs and also the body weights were averaged for each 50 gm increase in body weight, or for each 10 mm increase in body length and these averages were used in determining the formulae and also in calculating the average percentage deviations of the calculated from the observed averages.

TABLE 1

Weights and percentage weights of the eyeballs

	AVERAGE	COEFFICIENTS OF VARIATION
Eyeballs (grams)	0.9885 ± 0.0052	7.78 ± 0.37
Eyeballs (% of Body W.)	0.1536 ± 0.0010	9.76 ± 0.47

WEIGHTS AND PERCENTAGE WEIGHTS

The average weight in grams and the average percentage weight of the two eyeballs with reference to the body weight and the coefficient of variation are shown in table 1 with their probable error. This table shows that the weights in grams are less variable than the weights as percentages of the body weight, as was reported for both the brain and the spinal cord in these guinea pigs. In other words, the eyeballs, brain and cord vary less than the total body weight and also are more or less independent of the variation in the body weight. The weights of the eyeballs are slightly more variable than the weights of the brain (6.80 ± 0.33) but less variable than the total body weight with a coeffiicent of variation of 12.47 ± 0.60.

The averages given in table 1 are for average sized guinea pigs and to better estimate the weight of the two eyeballs from any guinea pig within the limits of body weight found in these specimens, empirical formulae have been found. The curves with the individual cases are not shown here as they are straight lines and the cases are only moderately scattered around the lines.

The empirical formula for determining the weight of the two eyeballs from body weight, with both weights in grams and from 400 to 900 gm of body weight is:

$$Y = 0.0006\ X + 0.5966.$$

Y represents the weight of the two eyeballs and X, the body weight, with both weights in grams. The average percentage deviation of the calculated from the observed averages was 1.23%.

The empirical formula for the weight of the two eyeballs when plotted on body length between 265 and 345 mm of body length is:

$$Y = 0.0028\ X + 0.1238.$$

Again, Y represents the weight of the two eyeballs in grams and X, the body length measured in millimeters. The average percentage deviation of the calculated from the observed values for this formula is 0.81%.

Bessesen and Carlson ('23) give a formula for finding the weight of the two eyeballs from body weight and their formula was used to calculate the weights of the eyeballs in this series. The average percentage difference between the values thus calculated and the observed values was 4.05%. This is not a bad fit, but not as good as the straight line formula given above. The curve plotted from their formula was too low at first and then toward the end it rose too rapidly. It was a second degree equation and thus one would expect a more rapid rise toward the end. The weight of the eyeballs for the average body weight of this series of guinea pigs was calculated by means of the formula given by Bessesen and Carlson and it came out 0.9620 gm or but 0.0265 gm less than the average of the weights of the two eyeballs, again showing that the formula for Minnesota guinea pigs fits the guinea pigs of Kansas very well.

In the list of the weights of the eyeballs, as given by Crile and Quiring ('40), one finds a wide range of weights, for example the eyes of the axis deer weigh 34.4 gm and the eyes of the collared peccary weigh but 10.2 gm and yet their percentages of the body weight are respectively, 0.039 and 0.35. The two eyeballs of the stallion weigh 136.7 gm and yet they form but 0.0215% of the body weight. The weights of the eyeballs of different species vary a great deal.

Oppenheim ('47) lists several animals and gives the weight of the eyeballs in grams and then the percentage weight of the eyeballs with reference to the weight of the brain. These percentages vary from 1.13 for man to 60.22 for the rabbit and 108.82% for the chicken. In this series of guinea pigs the eyeballs form 24.67% of the brain weight and 35.52% for the series of adult cats (Latimer, '38).

The percentages of the weights of the two eyeballs of the total body weight was determined for each specimen and the averages of these percentages are given in table 1. To better determine the percentage for any body weight or body length between 400 and 900 gm of body weight and 265 and 345 mm of body length, empirical formulae were determined by the method of least squares.

The percentage weight of the two eyeballs may be estimated from the gross body weight by the following formula:

$$Y = 0.2388 - 0.00013\ X.$$

In this formula Y represents the percentage weight of the two eyeballs and X, the body weight in grams. The average percentage deviation of the calculated from the observed values is 1.26%. These cases were all very closely grouped around the straight line. It will be shown later on that the highest correlation with the weights of the eyeballs is with the body weight and the close approximation of these cases to the regression line when plotted against body weight would bear this out. This curve and the following are not reproduced here as they are straight lines and the cases are closely grouped about the line.

The formulae for the percentage weight of the two eyeballs plotted on body length is as follows:

$$Y = 0.4117 - 0.00083\ X.$$

In this formula, Y represents the weights of the two eyeballs as percentages of the body weight, plotted on body length measured in millimeters. The average percentage deviation of the calculated from the observed values for this formula was 1.82%. This shows that there was a little more variation when plotted on body length and the correlations will show a lower correlation between eyeball weight and body length than between eyeballs and body weight.

Both of these lines started at a higher level and decreased slightly in the larger guinea pigs. We have data for three groups of guinea pigs: one group of two males with an average body weight of 361 gm; another group of 10, with an average weight of 456 gm (Crile and Quiring, '40) and this group with an average body weight of 649.44 gm. The percentage weights of the eyeballs in the three groups are respectively, 0.258, 0.184 and 0.154%. The two groups given by Crile and Quiring are both lighter in body weight than this group, and in general the eyeballs are relatively larger in smaller animals, or in younger animals (Latimer, '48).

The table of body weights and the weights of the eyeballs given by Crile and Quiring ('40) was used to calculate the percentages of the eyeballs to the total body weight for all adult male animals. Not only was there a wide range in the weights in grams of the eyeballs but the percentages varied also, although to a lesser degree. The total range in the percentages of the eyeballs with reference to body weight was from 0.00175% for the elephant to 2.946% for the sparrow hawk. There was a wide range in each of the groups. The averages of the percentages for each group were found and again there was quite a difference. With some exceptions it seemed that the larger animals had relatively smaller eyes.

This holds true for the following groups: Ungulates (0.069%), Pinnepedia (0.082%), Anthropoids (0.083%), Elasmobranchs (0.232%), Bony fishes (0.689%), and Birds (1.083%). These have a decreasing average body weight while the percentages of the eyeballs, given in parenthesis, increase. The two groups which did not fit into this order were the Carnivora with an average body weight of 44.6 kg and an average percentage weight of the eyeballs of 0.146, and the Rodents with an average body weight of 206.3 gm and 0.217% for the eyeballs. The carnivora had too large eyeballs for their average body weight but this might be explained by their night vision. The rodents should have had the relatively largest eyes according to this scheme but they did not. It is well known that young animals have relatively larger eyeballs than the adults of the same species for the eyeballs like the brain are precocious in their development. It appears that in general one might expect to find relatively larger eyes in smaller animals but of course the habits of the animals must be considered.

CORRELATIONS

The correlations between the weights of the two eyeballs and the 13 dimensions of these guinea pigs are shown in table 2. The two best correlations are with body weight and body

TABLE 2

Correlations with the eyeballs

Body weight	+ 0.643 ± 0.040
Brain weight	+ 0.233 ± 0.064
Cord weight	+ 0.471 ± 0.052
Body length	+ 0.515 ± 0.050
Cord length	+ 0.492 ± 0.051
Prosencephalon	+ 0.209 ± 0.065
Mesencephalon	+ 0.249 ± 0.063
Cerebellum	+ 0.129 ± 0.066
Medulla	+ 0.239 ± 0.064
Hypophysis	+ 0.204 ± 0.065
Thyroid	+ 0.208 ± 0.065
Suprarenals	+ 0.283 ± 0.062
Testes	+ 0.479 ± 0.052

length, and these are the only significant correlations in this table. Next in order is the correlation with cord length and it is but slightly below 0.5. The lowest correlation is with the cerebellum. This is not only too low to be significant, but in addition its probable error is a little over half as large as the correlation. The average of the correlations with the parts of the central nervous system is but 0.289, or just half the average of the correlations with body weight and body length. The average of the correlations with the endocrine glands is but slightly higher or 0.294.

The correlations of the weights of the eyeballs with body weight and body length in the guinea pig, turkey (Latimer and Rosenbaum, '27) and adult cat (Latimer, '47) are all significant, but they are the lowest in the guinea pig. The correlations between body weight and eyeball weight are higher in all cases than with the body length. The average of the correlations between the eyeballs and the endocrine glands is much higher in the cat, or a correlation of 0.537. The correlation between the eyeballs and the hypophysis in the adult cat is but 0.048 or but 23.5% of the correlation in the guinea pig.

On the whole, the eyeballs in the guinea pig are not as well correlated with body weight, body length, the brain and spinal cord weights and the weights of the 4 endocrine glands as they are in the turkey and the adult cat. The coefficient of variation of the weights of the eyeballs in the guinea pig is less than in the cat, or 7.78 in the guinea pig compared to 10.62 in the male cat. Do these lower correlations with the guinea pig eyeballs mean that the eyes in the guinea pig are less variable in weight than in the cat or turkey (coefficient of variation of 8.02) and that they do not vary in weight with these other organs as they do in the cat or turkey? We may conclude that there is less variability in the weights of these parts in the guinea pig but also a smaller amount of relationship between these parts than in some animals which vary more in total size but still retain a more constant interrelationship between the parts.

SUMMARY

The two eyeballs average 0.99 gm. They are less variable in weight than the total body weight or the weight of the spinal cord, but more variable than the weight of the brain.

They average 0.15% of the body weight, and 24.67% of the brain weight. Their percentage weights are more variable than the weights in grams.

Empirical formulae are given by means of which the weights in grams or the percentage weights may be determined from either the body weight or body length.

The weights of the eyeballs are not well correlated with the other dimensions. They are significantly correlated only with the body weight and body length. The correlations with 7 dimensions of the central nervous system, and with the weights of 4 endocrine glands are all positive but none are high enough to be significant.

REFERENCES

Bessesen, A. N., and H. A. Carlson 1923 Postnatal growth in weight of the body, and of the various organs in the guinea pig. Am. J. Anat., *31:* 483–521.

Crile, George and D. P. Quiring 1940 A record of the body weight and certain organ and gland weights of 3690 animals. Ohio J. Sci., *40:* 219–259.

Jackson, C. M. 1913 Postnatal growth and variability of the body and of the various organs in the albino rat. Am. J. Anat., *15:* 1–68.

Latimer, H. B. 1925 The relative postnatal growth of the systems and organs of the chicken. Anat. Rec., *31:* 233–253.

——— 1927 Correlations of the weights and lengths of the body, systems and organs of the turkey hen. Anat. Rec., *35:* 365–377.

——— 1938 The weights of the brain and of its parts, of the spinal cord and of the eyeballs in the adult cat. J. Comp. Neur., *68:* 395–404.

——— 1947 Correlations of organ weights with body weight, body length and with other weights in the adult cat. Growth, *11:* 61–75.

——— 1948 The prenatal growth of the cat. XVI. Changes in the relative weights of the organs. Growth, *12:* 123–144.

——— 1950 The weights of the brain and of its parts and the weight and length of the spinal cord in the adult male guinea pig. J. Comp. Neur., *93:* 37–52.

Latimer, H. B., and J. A. Rosenbaum 1927 A quantitative study of the anatomy of the turkey hen. Anat. Rec., *34:* 15–23.

Oppenheim, S. 1947 Metrische und deskriptive Merkmale des menschlichen und tierschen Auges. Tab. Biol., *22:* 54–153.

SCAMMON, R. E., AND E. L. ARMSTRONG 1925 On the growth of the human eyeball and optic nerve. J. Comp. Neur., *38:* 165–219.

SNEDECOR, G. W. 1938 Statistical methods. Collegiate Press, Ames, Iowa.

STEINDORFF, K. 1947 Deskriptive Anatomie des Auges der Wirbeltiere und des Menschen. Tab. Biol., *22:* 166–297.

STAINING OF STRATIFIED SQUAMOUS EPITHELIUM OF MUCOUS MEMBRANES AND SKIN OF MAN AND MONKEY BY THE PERIODIC ACID-SCHIFF METHOD[1]

G. B. WISLOCKI, D. W. FAWCETT AND E. W. DEMPSEY[2]
Department of Anatomy, Harvard Medical School, Boston, Massachusetts

THIRTEEN FIGURES

The cells of stratified squamous epithelium are separated by intercellular spaces or clefts filled with some sort of cementing ground substance. The cells of the stratum germinativum or spinosum are united by protoplasmic processes termed intercellular bridges which cross the intercellular clefts. Most histologists agree that the bridges are integral parts of the cells. However, Chambers ('24) has expressed doubt regarding their existence in living cells, considering them as "either artefacts or indications of a fibrous structure of the intercellular cement or a combination of the two." Observations reported recently on the intercellular bridges of vaginal and gingival epithelium stained by sudan black B (Wislocki, Bunting and Dempsey, '50) and Baker's acid hematein method for phospholipids (Wislocki, '51) indicate that these structures contain lipid and are definitely parts of the cells.

The present investigation concerns staining of the intercellular material and the cell surfaces in the outer layers of the stratified squamous epithelium of mucous membrane and epidermis of man and rhesus monkey with the Schiff reagent after periodic acid oxidation. The staining under consideration occurs after preliminary treatment of the sections with saliva and consequently appears to be attributable to some carbohydrate-containing compound other than glycogen.

---

[1] The work was aided by grants from the American Cancer Society: (1) on the recommendation of the Committee on Growth of the National Research Council, and (2) from an Institutional Grant to Harvard University.

[2] Present address: Department of Anatomy, Washington University School of Medicine, St. Louis 10, Missouri.

MATERIAL AND METHODS

The material comprised pieces of vagina, gingiva, esophagus, tongue, lip and skin taken from two freshly killed rhesus monkeys of about one year of age, as well as pieces of vagina and skin obtained from human subjects. The specimens of human vagina were removed either from the portio vaginalis or from the so-called vaginal cuff of uteri removed in total hysterectomy. Three of the uteri were removed during the late proliferative phase of the cycle, one during the early secretory phase and one during the third month of gestation. The pieces of monkey skin were taken from sole of foot, thigh, abdomen, vulva, lip and ear. Two pieces of human skin were obtained from sole of the foot and the plantar surface of the great toe.

The pieces of tissue were fixed routinely in Rossman's fluid (abs. alc. sat. with picric acid, 90 cm^3; formaldehyde [40%], 10 cm^3), but a few blocks were also fixed in Zenker's and Orth's fluids. The blocks were imbedded in paraffin and the sections were cut at 6 μ. In one instance, a piece of human vaginal wall was immersed in saliva for one and one-fourth hours before fixing it in Rossman's fluid.

The deparaffinized sections were stained by the periodic acid-Schiff method according to the procedure of McManus ('46). To eliminate glycogen and thereby distinguish it from the positively reacting substances, half of the sections were exposed to saliva for two hours at room temperature before staining. No counterstain was employed. The sections were dehydrated, cleared and then mounted in clarite.

In addition to the above, vaginal smears were obtained from a woman with a normal menstrual history. The smears were fixed, without initial drying, in a solution of equal parts of ether and 95% alcohol. After leaving them overnight in the fixative, they were stained by the periodic acid-Schiff method with and without saliva treatment, and then lightly counterstained with Harris' hematoxylin.

OBSERVATIONS

The vagina. In the stratified epithelium of the vaginal wall of man and rhesus monkey large quantities of periodic acid-Schiff-stained material are present. Much of this, particularly that occurring in the cytoplasm of the cells, is glycogen. However, it does not appear to have been noted before that, after the sections have been exposed to saliva, there is a considerable residual staining, seemingly involving the intercellular material and the cell surfaces of the epithelium of the so-called stratum superficialis (cornified or squamous zone) and of the outer part of the subjacent stratum spinosum. This is illustrated in figures 1 and 2 showing the human vaginal mucosa stained by the periodic acid-Schiff reagents before and after exposing sections to saliva for two hours, as well as in figures 3 and 4 depicting a similar result in the vaginal mucosa of an immature rhesus monkey. From the difference in distribution of stained material before and after digestion with saliva, it is apparent that glycogen is responsible for the bulk of the intracellular staining. However, there is a residual reaction after exposure to saliva, which is associated with the intercellular material and cell surfaces of the outer layers of the epithelium.

This residual reaction imparts a red color of variable intensity to the superficial layers of the vaginal epithelium. The width of the reactive zone appears to depend upon the relative thickness of the so-called stratum superficialis. This is demonstrated by comparing the very wide zone which gives the reaction in the human vagina of the late proliferative phase (fig. 2) with the relatively narrow zone in the immature monkey vagina (fig. 4). We have not had access to sufficient material from different stages of the human menstrual cycle to define the variation in width and intensity of the reaction which may occur in the various phases. The specimen at our disposal from the early secretory stage was only slightly less colored than the one illustrated from the late proliferative phase. The specimen from the third month of gestation, however, was distinctly less deeply stained.

The saliva-resistant substance (or substances) responsible for the observed residual staining appears to be situated in the intercellular spaces surrounding the prickle cells of the outermost portion of the stratum spinosum as well as in the intercellular spaces and on the cell surfaces of the superficialis. Upon examination of the sections with the oil immersion lens further details of the reaction become apparent (fig. 7). In the outermost zone of prickle cells, where the staining commences, the reactive substance seems to be located in the intercellular spaces alternating with the intercellular bridges. In the supervening superficialis the intercellular bridges are no longer apparent, although the intercellular clefts are wider and more evident. The intercellular clefts are filled with a red stained substance in which numerous deeply stained granules are apparent. As illustrated in figure 7, the granules seem to be located largely on the cell surfaces or possibly even in the cell membranes, accentuating the outlines of the squamous cells. Thus the eye encounters parallel rows of deeply stained granules with a less deeply stained, rather homogeneous intercellular substance located between them. In the most superficial layer of the vaginal epithelium the squama become extremely flattened and the intercellular spaces are much narrower than in the deeper layer. As a consequence, the intercellular cementing substance diminishes in amount and the granules become reduced in number and size, with a corresponding diminution in amount of staining.

In view of the observations reported above upon staining of sections of vaginal epithelium before and after treatment with saliva, it became of interest to examine the staining reactions of desquamated vaginal epithelium as encountered in smears. Figure 12 illustrates the intensity of the periodic acid-Schiff reaction without the use of saliva, whereas figure 13 shows a similar field after exposing the smear to saliva for two hours. It will be observed in the first smear that the desquamated cells are deeply stained and heavily laden with glycogen (fig. 12), whereas after saliva treatment, except for occasional dark cells, the squama are quite pale. The occasional dark staining cells appear to be ones that have somehow escaped the action of the saliva and hence have preserved their glycogen.

These findings in the vaginal smears seemed so interesting that a further step was taken to attempt to relate the appearance of the desquamated cells to observations made on sectioned material. For this purpose two further blocks were taken from the vaginal wall of a specimen in the late proliferative phase. Sections from the first block were stained, both without using saliva (fig. 9) and after exposure to saliva for two hours (fig. 11). The differences between the two are striking. The second block was immersed in saliva for one and one-fourth hours *before* placing it in fixative and sectioning it. As a consequence, the saliva had to penetrate the intact block and not merely a thin histological section. The differences resulting from these two modes of penetration of the saliva are apparent in comparing figures 10 and 11. In figure 10, where the block was exposed to saliva befor making the sections, the superficial layers of cells show a marked disappearance of glycogen, whereas the deep layers show retained glycogen. However, the persistence in the superficial layers of a few deeply stained glycogen-bearing cells, two of which are clearly evident in the photograph, is of interest. Upon comparing this section with the vaginal smear exposed to saliva (fig. 13), it is apparent that the occasional dark stained cells in the smear correspond approximately in number and depth of staining to those dark saliva-resistant cells observed near the surface of the vaginal epithelium. Furthermore, upon turning to figure 11, where the cells have been cut open, by sectioning them before exposure to saliva, no such deeply stained cells are encountered. From these observations the conclusion is drawn that occasional squama of the superficial layer of the vagina are so heavily encapsulated that saliva does not penetrate them, such squama accounting for the occasional darkly stained cells persisting in the vaginal smears despite prolonged exposure to saliva. In sections which have been cut before treatment with saliva (fig. 11) all of the cells seem to have been opened up, as a consequence of which glycogen-containing dark cells of the character seen in figure 10 are not apparent. Moreover, it follows from the above that the residual staining, visible in figure 10, is probably attributable to a substance other than glycogen. The longest we have exposed a piece of vagina to saliva was for three and one-fourth hours (one and one-fourth hours as a fresh block and two more hours after it was sectioned). Upon staining this section, the residual periodic acid reaction was quite similar in intensity to the reaction shown in figure 11, a result which suggests that all of the glycogen had been digested.

The esophagus. In monkey's esophagus an intense staining of the intercellular substance in the superficial squamous layer and outer part of the stratum spinosum is visible, following application of the periodic acid-Schiff reagents (fig. 8). The epithelium of the esophagus contains mere traces of glycogen, compared with vagina, so that the specimens before and after exposure are almost identical. The esophageal stratified epithelium resembles that encountered in the vagina, except that the superficial squamous layer is very broad and the intercellular material forms relatively narrow stripes or lines extending between the cells. As in the vaginal epithelium, the intercellular substance stains quite intensely. Under high magnification, the material is observed to be finely granular, especially in the outer part of the stratum spinosum where stained intercellular substance is first encountered. However, since the intercellular clefts are narrower and the granules much finer, the double-contoured appearance of the intracellular spaces is not as evident as in the vaginal epithelium.

The gingiva. Monkey's gingiva presents a similar picture (fig. 6). After ridding the sections of some glycogen which is present, by immersion in saliva, a fine-grained, deeply stained granular deposit persists between the cells. In the outer layers of squamous cells, the granules appear to be located mainly on the cell surfaces, frequently producing parallel lines of reactive dots.

The intensity and amount of staining in the gingiva are subject to local variations. No explanation for this is apparent, although it is noted that Orban ('44) has recorded local variations in the epithelium of the gingiva in reference to the degree to which granular and cornified layers are differentiated.

The tongue and mucous membrane of the lips. The stratified epithelium of these structures, obtained from two monkeys, shows similar staining with the periodic acid-Schiff reagents, although here again the staining varies locally in intensity with no apparent reason. If the maximal staining of the residual, saliva-resistant material in the vagina and esophagus, as described in previous passages, is assigned an arbitrary value of four, then gingival staining would have a value of two or three, while the tongue and mucous membrane of the lip would exhibit values diminishing from two down to traces.

The skin. The staining of the intercellular material in the outer squamous layers of the skin of man and rhesus monkey by the periodic acid-Schiff reaction is very slight compared to the mucous membranes. At first we considered writing the skin off as completely negative, but upon repeated examination it seemed apparent that a similar, although quite faint, staining usually occurred in the epidermis. The maximum degree of staining which we have encountered in skin is illustrated in figure 5, a specimen obtained from the plantar surface of a human toe. There is a definite reaction in the intercellular spaces of the stratum spinosum and between the very flattened cells of the stratum corneum, while the reaction in the intervening granulosum is distinctly less intense. By comparison with the mucous membranes, on the numerical scale which we have suggested above, the epithelium in this specimen has an approximate value of one. By this token, skin from various sites in man and monkey ranges from one (human sole, monkey sole), through barely recognizable traces (monkey thigh, lip and vulva), down to zero (monkey abdomen and ear). The striking difference in degree of staining between mucous membrane and skin is well illustrated by a section which shows the deeply stained outer squamous epithelium of the monkey's vaginal wall passing by sudden transition into the very nearly unstained epidermis of the genital vestibule. In a section through the muco-cutaneous junction of a monkey's lip this difference is much less marked.

The effect of fixation on the reaction. Pieces of mucous membrane and skin were routinely examined after fixation in a mixture of alcohol, formalin and picric acid (Rossman's fluid). The results obtained were those described in the preceding passages.

Orth's fluid was also tried because it has been recommended by Leblond ('50) as the fixative of choice for preserving periodic acid-Schiff positive material. Our use of this fixative on a number of tissues in other studies has confirmed Leblond's experience. Nevertheless, in the present instance, involving fixation of skin and mucous membranes, preservation and staining were not as good after fixation in Orth's as in Rossman's fluid. Zenker's fluid was also tried, but, although the staining seemed better than after Orth's fluid, it was not quite as intense as after Rossman's fixative.

DISCUSSION

A substance, or substances, in the stratified squamous epithelium of various mucous membranes and epidermis gives a periodic acid-Schiff reaction after removal of glycogen from the tissue sections. It is generally agreed that compounds containing 1, 2 glycol linkages are oxidized by periodic acid with the formation of aldehyde groups which are capable of reacting with Schiff's leucofuchsin reagent (Hotchkiss, '48). Various types of compounds have been found capable of giving this reaction, but, since the present material was exposed to fat solvents, as well as to aqueous media in the course of preparation, the number of possible reactive substances remaining in the sections is considerably reduced. It has been suggested by a number of writers, including Leblond ('50), that provided glycogen has been removed, the material reacting in sections prepared and treated in the present manner is a mucopolysaccharide or a mucoprotein, although Lillie ('50) raises certain objections to such a specific interpretation, being willing to say no more than that the reaction indicates adjacent glycol or hydroxyamino groupings.

Quite aside from the question of the nature of the reactive carbohydrate, the present observations are of interest in several respects. A reaction has been observed involving saliva-resistant material located in the intercellular spaces and on, or in, the cell surfaces of the superficial epithelial layers of mucous membranes and epidermis. The reaction is generally much more intense in mucous membranes than in skin, a difference for which no explanation can be offered. One can point out merely that the outer layers of skin are subject to dessication and contain keratin, whereas mucous membranes are moist and are believed normally to contain little or no keratin. Hence, the periodic acid-Schiff reactive material may be inversely related to the amount of keratin in a given epithelium, a question which should be investigated by suitable methods. In connection with the possibility of such a relationship it is interesting to recall that the stratified squamous epithelial lining of the guinea pig's vagina during the estrous cycle alternately produces mucus-containing cells and cornified cells, the mucus being an acid mucopolysaccharide which contains sulfate groups, and the cornified cells being characterized chemically by the presence of sulfur as SH and S-S groups. In the enamel organ of developing teeth Wislocki and Sognnaes ('50) have called attention to a similar production by epithelial cells, first of an acid mucopolysaccharide and later of a substance containing SH or S-S groups. Similarly in vitamin A deficiency, as well as in other pathological conditions, metaplastic keratinization of mucous membranes has been frequently reported.

The origin of the saliva-resistant periodic acid-Schiff retive material can merely be surmised. Several possibilities exist. It may be a local metabolic product of the epithelial cells, formed in or on their surfaces and discharged or secreted into the intercellular spaces. On the other hand, it may have reached its destination by diffusion of an unstained precursor from the underlying lamina propria outward through the intercellular spaces.

In the case of the vagina, where the saliva-resistant, reactive granules in the stratum superficialis are relatively large and intensely stained, a remote possibility exists that they may be composed of a glycogen-protein complex in which the glycogen is so firmly bound that it is not readily and completely destroyed by even prolonged exposure to saliva. This contingency, however, does not seem likely in view of the observation that the esophagus, which contains little glycogen, is very similar in appearance.

Occasional intensely stained, saliva-resistant epithelial cells are encountered in vaginal smears. These squama appear to possess a cell membrane which is impermeable to saliva, even upon prolonged exposure. Their origin from squamous cells in the stratum superficialis has been demonstrated, and it has also been shown that the glycogen in them becomes accessible to saliva as soon as the cells are cut open.

The bulk of the desquamated vaginal epithelial cells is stained a pink color after exposure to saliva, being relatively pale in contrast to the more deeply stained cementing substances enclosing the squama while they are still imbedded in the stratum superficialis. Since the cementing substance is probably a mucopolysaccharide, it is possible that it is digested in the process of exfoliation by hyaluronidase or some other enzyme present in vaginal secretion. A degree of support is lent to this surmise by the observation that the exfoliated cells are relatively pale. We have examined the cells of vaginal smears in only one individual with a supposedly normal menstrual history. A survey of the exfoliated cells, stained by the periodic acid-Schiff procedure, in various phases of the menstrual cycle, as well as in pathological conditions, might disclose further observations of value.

SUMMARY

In the intercellular spaces and on the cell surfaces of the epithelium of the outer layers of stratified squamous epithelium of mucous membranes and skin of man and monkey, material is present which stains with the periodic acid-Schiff method. This material, which resists digestion by saliva and hence is not glycogen, takes the form of homogeneous substance filling the intercellular spaces and of granules located on, or possibly in, the cell membranes. The granules stain more intensely than the homogenous element. These reactions are intense in vaginal and esophageal epithelium and variably strong in gingiva and in the mucous membranes of tongue and lip. In epidermis, on the other hand, staining ranges from faint down to no appreciable reaction. Possible reasons for the marked difference in staining of mucous membranes and skin are briefly discussed and the suggestion is offered that the amount of periodic acid-Schiff reactive material may vary inversely to the amount of keratin present in a given epithelium.

REFERENCES

CHAMBERS, R. 1924 The physical structures of protoplasm as determined by micro-dissection and injection. In: Cowdry's General Cytology, 235–309.

HOTCHKISS, R. D. 1948 A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem., *16:* 131–141.

LEBLOND, C. P. 1950 Distribution of periodic acid-reactive carbohydrates in the adult rat. Am. J. Anat., *86:* 1–49.

LILLIE, R. D. 1950 Further exploration of the HIO_4-Schiff reaction with remarks on its significance. Anat. Rec., *108:* 239–253.

McMANUS, J. F. A. 1946 Histological demonstration of mucin after periodic acid. Nature, *158:* 202.

ORBAN, B. 1944 Oral Histology and Embryology. C. V. Mosby, St. Louis, Mo., 267–268.

WISLOCKI, G. B. 1951 The staining of the intercellular bridges of the stratified squamous epithelium of the oral and vaginal mucosa by sudan black B and Baker's hematein method. Anat. Rec. (Abstract), *109:* 128–129.

WISLOCKI, G. B., AND R. F. SOGNNAES 1950 Histochemical reactions of normal teeth. Am. J. Anat., *87:* 239–276.

WISLOCKI, G. B., H. BUNTING AND E. W. DEMPSEY 1950 The chemical histology of the human uterine cervix with supplementary notes on the endometrium. In: Menstruation and its Disorders, edited by Earl T. Engle. Charles C. Thomas, Springfield, Illinois.

PLATE 1

EXPLANATION OF FIGURES

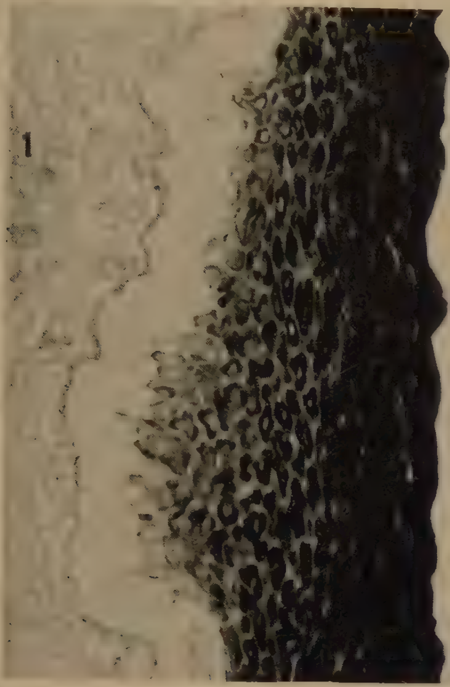
1 Section of human vaginal mucosa stained with the periodic acid-Schiff reagents. Late proliferative phase. Rossman's fixative. × 300.

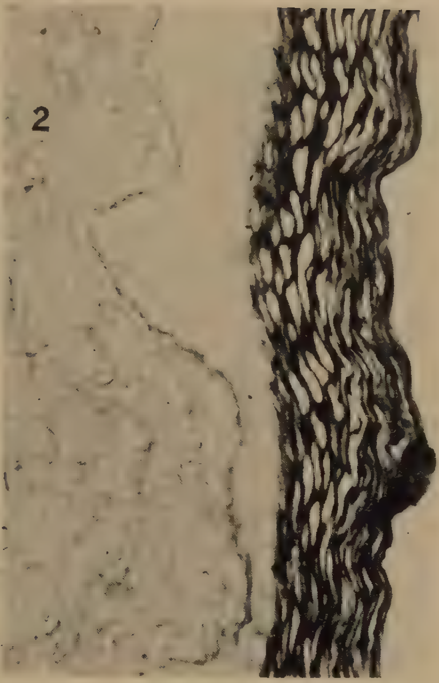
2 A section of the same as in figure 1, after immersion for two hours in saliva. Observe the intensely stained saliva-resistant residual material, located in the intercellular spaces of the superficial layer and the outer part of the stratum spinosum. Note the empty, unstained cellular spaces from which the glycogen has been removed, and compare with figure 1 in which glycogen is still present. Compare also with figure 7 which shows a portion of this section highly magnified. × 300.

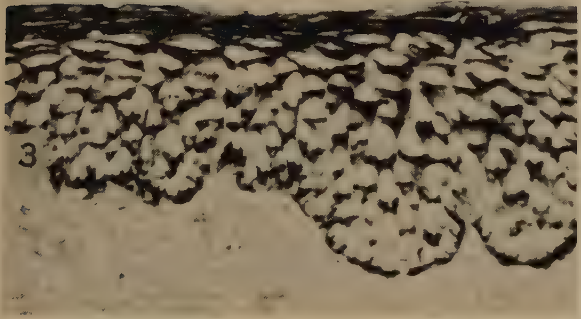
3 Section of vaginal mucosa of an immature rhesus monkey stained with the periodic acid-Schiff reagents. Rossman's fixative. × 300.

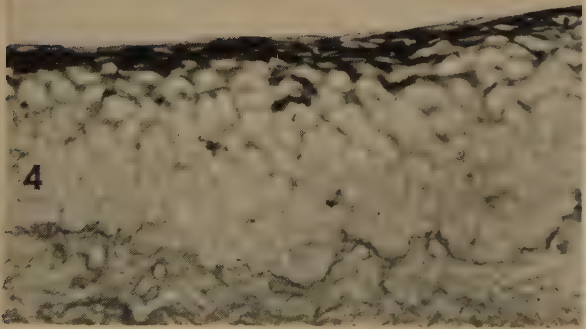
4 A section of the same as figure 3 after removal of the glycogen by saliva. Observe the residual periodic acid-Schiff reaction and compare the appearance with that of figure 3 in which glycogen is present. × 300.

5 A section of human skin from the plantar surface of a great toe. Rossman's fixative. This illustrates the maximal amount of periodic acid-Schiff reaction encountered in epidermis. There is a faint reaction in the intercellular spaces of the stratum spinosum and a somewhat deeper reaction in the less plainly defined intercellular spaces of the stratum corneum. × 250.


1


2


3


4

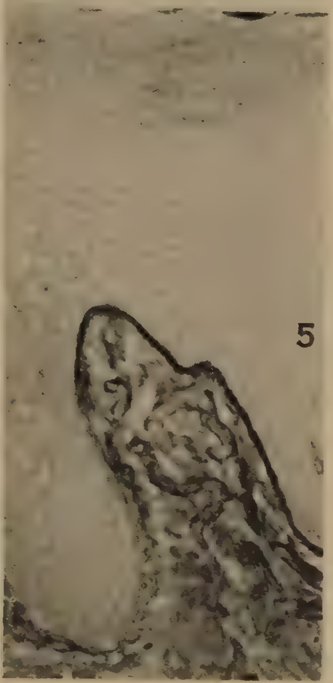

5

PLATE 2

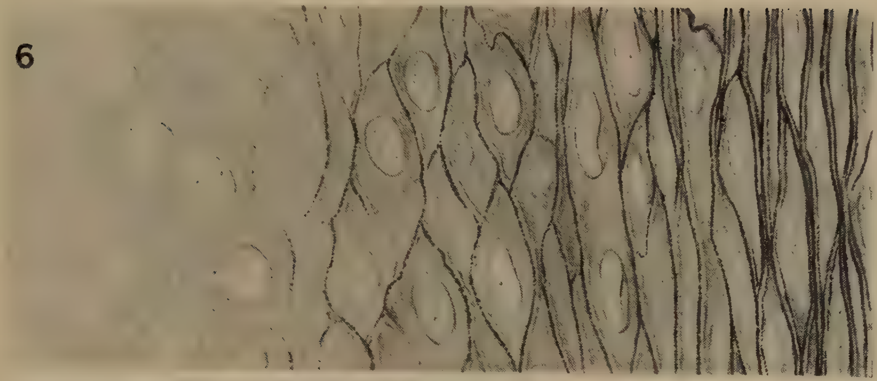
EXPLANATION OF FIGURES

6 Gingiva of rhesus monkey. Rossman's fixative. Section immersed for two hours in saliva to remove glycogen. Periodic acid-Schiff stain. Observe the periodic acid-Schiff reactive material in the intercellular spaces of the stratum corneum and the outer part of the stratum spinosum. × 7 oc., × 90 obj.

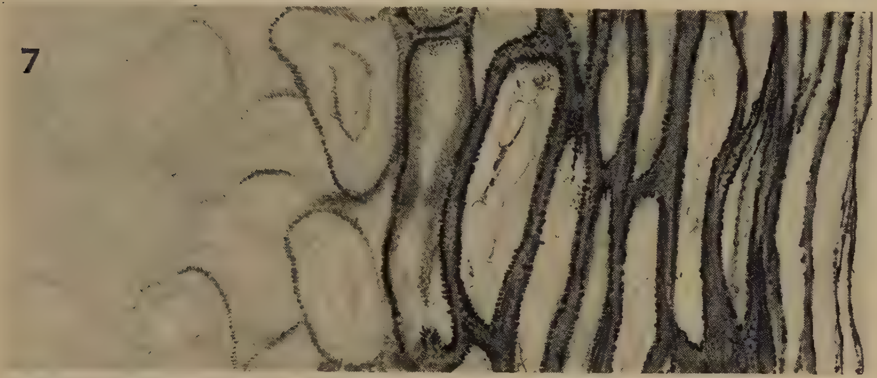
7 Human vagina. Early secretory phase. Rossman's fixative. Section immersed for two hours in saliva. Periodic acid-Schiff stain. Observe the periodic acid-Schiff reactive material in the intercellular spaces of the stratum corneum and the outer part of the stratum spinosum. The figure illustrates a typical portion of figure 2 highly magnified. × 10 oc., × 90 obj.

8 Esophagus of rhesus monkey. Rossman's fixative. Section immersed for two hours in saliva. Periodic acid-Schiff stain. × 200.

6



7



8

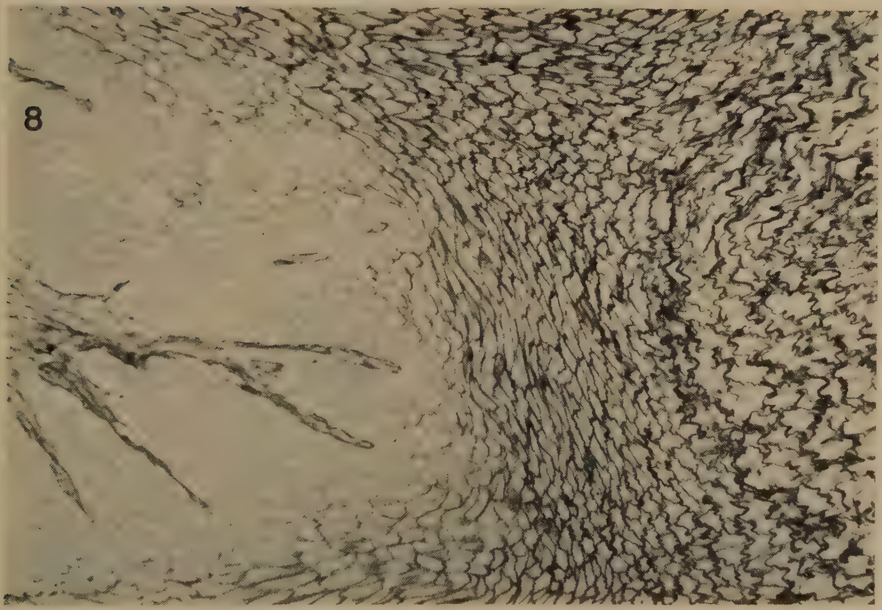


PLATE 3

EXPLANATION OF FIGURES

9 Section of human vaginal mucosa. Proliferative phase. Rossman's fixative. Periodic acid-Schiff stain. Compare with a vaginal smear similarly stained (fig. 12). × 250.

10 Section of the same vagina, from a block of tissue which was immersed in saliva for one and one-fourth hours before it was fixed. Observe that the saliva penetrated the superficial layer of the vaginal epithelium, sparing occasional glycogen-laden cells, but not the deeper part. There occasional, darkly stained superficial cells seem to account for the occasional, similarly stained cells in vaginal smears after saliva treatment (fig. 13). × 250.

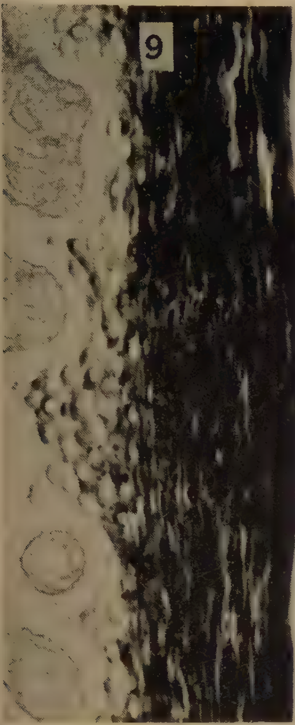
11 Section from the same specimen as figure 9, stained after immersion of the section for two hours in saliva. Compare with figures 7 and 9, noting the degree and distribution of the saliva-resistant, residual reaction. Compare with figure 10, in which the entire block, but not the section, was exposed to saliva. Observe that, unlike figure 10, there are no darkly stained glycogen-laden cells in the superficial layers, a result attributed to the fact that in cutting the section all of the cells were opened, permitting entrance of saliva to even the occasionally very resistant ones. × 250.

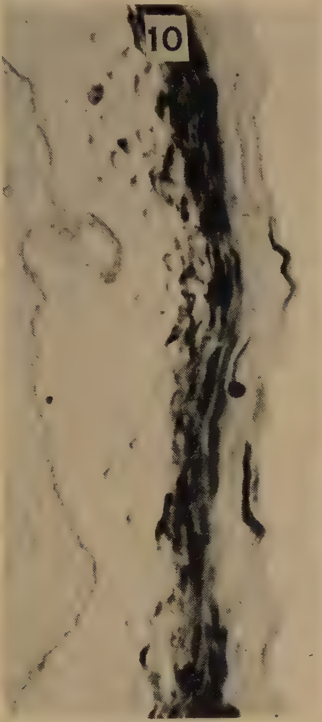
12 A smear of human vaginal fluid. Proliferative phase. Alcohol and ether fixation. Periodic acid-Schiff reaction. Compare with figure 9. × 200.

13 A similar smear, which was exposed for two hours to saliva before staining it. Compare with figure 10. × 200.

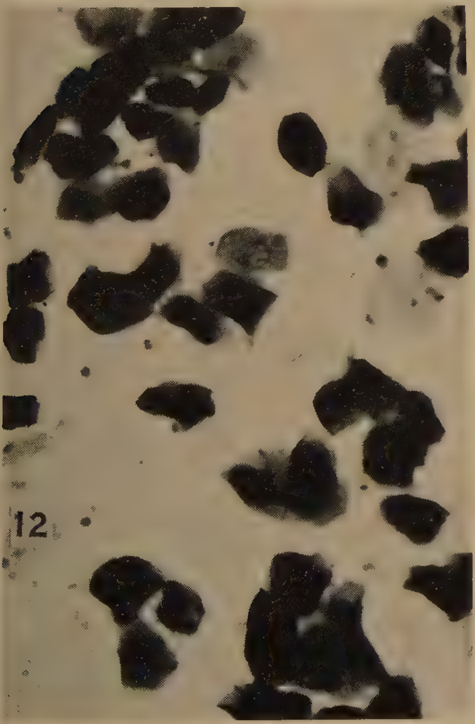
SCHIFF REACTION OF STRATIFIED EPITHELIUM
WISLOCKI, FAWCETT AND DEMPSEY

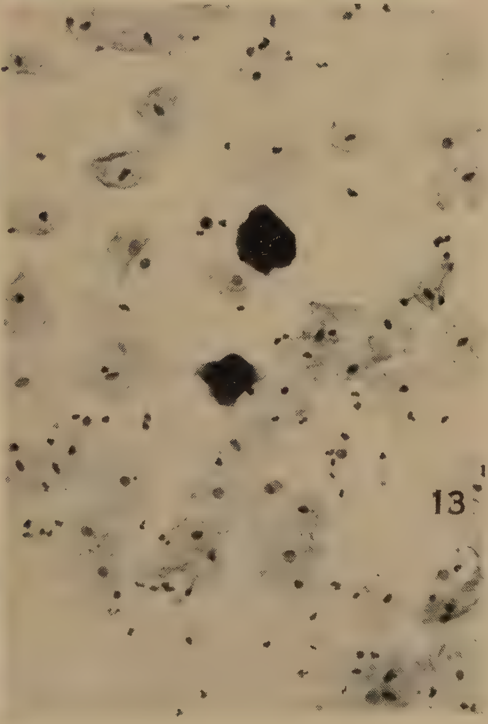
PLATE 3


9


10

11


12


13

PROCEEDINGS

OF THE

AMERICAN ASSOCIATION OF ANATOMISTS

Sixty-fourth Annual Meeting

Notice of 1952 Meeting

THE NEXT ANNUAL MEETING
OF THE AMERICAN ASSOCIATION
OF ANATOMISTS WILL BE HELD
IN PROVIDENCE, RHODE ISLAND,
AT THE INVITATION OF
BROWN UNIVERSITY,
ON MARCH 19, 20, AND 21, 1952.

~

A preliminary announcement with
details of the meeting will be mailed
to members of the Association about Dec. 1.
It may be necessary to set the deadline
for receipt of abstracts about
Jan. 1, 1952.

~

THE 1953 MEETING
IS TENTATIVELY PLANNED FOR
COLUMBUS, OHIO,
AT THE INVITATION OF
OHIO STATE UNIVERSITY,
ON APRIL 1, 2, AND 3, 1953.

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

SIXTY-FOURTH MEETING

Wayne University School of Medicine,
Detroit, Michigan, March 21, 22, and 23, 1951

The Association held its sixty-fourth meeting in Detroit, March 21, 22, and 23, at the invitation of Wayne University School of Medicine. The Local Committee consisted of Raymond Collings, Gaynor Evans, John Green, Gabriel Lasker, Gordon Scott, and Ernest Gardner, Chairman.

Registered Attendance:

	1951	*1950*		*1951*	*1950*
Association Members	347	290	Out of town	592	438
Non-members	270	182	Local	25	34
	617	472		617	472

Geographical Distribution of Registrants:

Alabama	6	Maryland	20	Pennsylvania	56
Arizona	0	Massachusetts	27	Rhode Island	9
Arkansas	1	Michigan	43	South Carolina	1
Colorado	4	Minnesota	17	South Dakota	3
California	15	Mississippi	4	Tennessee	11
Connecticut	9	Missouri	22	Texas	11
Delaware	0	Montana	0	Utah	2
D. of C.	6	Nebraska	9	Vermont	1
Florida	1	Nevada	0	Virginia	6
Georgia	10	New Hampshire	0	Washington	6
Idaho	0	New Jersey	1	West Virginia	3
Illinois	56	New Mexico	0	Wisconsin	20
Indiana	9	New York	64	Wyoming	0
Iowa	16	North Carolina	11	Canada	42
Kansas	13	North Dakota	2	Denmark	1
Kentucky	9	Ohio	22	Greece	1
Louisiana	11	Oklahoma	2	India	1
Maine	2	Oregon	3	Puerto Rico	2
				Location unknown	1

RÉSUMÉ OF SCIENTIFIC SESSIONS

The sixty-fourth session was opened by President Sam Clark with a general session in the Grand Ballroom of the Book-Cadillac Hotel on Wednesday morning, March 21. Dr. Clark announced the appointment of the Auditing Committee for 1951, H. Stanley Bennett, Arch E. Cole, and Raymond Collings, chairman. He further announced the appointment of the Nominating Committee for 1952, to consist of Edward Dempsey, Philip Armstrong, and Harold Davenport, chairman.

The general session consisted of ten papers selected by the Program Committee as having general interest. One paper was cancelled because of illness. Wednesday afternoon was devoted to 51 demonstrations held in the Science Building and Engineering Building of Wayne University. All other scientific sessions and the business session were held at the Book-Cadillac Hotel. There were two motion picture sessions Wednesday evening, with a showing of twelve films.

The remainder of the program consisted of four concurrent sessions mornings and afternoons of both Thursday and Friday, March 22 and 23. Two hundred and one papers were presented, six papers being cancelled. Sixty papers were read by title.

SOCIAL ACTIVITIES

On Wednesday afternoon, the wives of the staff of the Anatomy Department of Wayne University served coffee and doughnuts during the entire time of the demonstrations. This informal reception was not only greatly enjoyed by everyone, but deeply appreciated, as it alleviated the fatigue engendered by walking around the demonstration rooms.

The Smoker was held on Wednesday evening after the motion picture sessions in the new Veteran's Memorial Building. The attendance was 750, which is evidence of the success of the occasion.

On Thursday afternoon, the Department of Anatomy held an informal reception in the Medical School Building, giving the members an opportunity to see some of the laboratories. About 200 persons enjoyed the reception, and thanks are again due the wives of the department staff.

The Banquet on Thursday evening was attended by 321 persons. The Association was made welcome by Dr. David Henry, President of Wayne University, Gordon Scott, Dean of the School of Medicine, and Ernest Gardner, Professor of Anatomy. The principal after-dinner speaker was Dr. Joseph Hinsey, who spoke of the problems facing us in the current emergency.

MINUTES OF THE BUSINESS SESSION

March 22, 1951, 12:15 P.M.

The following items of business were transacted:

1. *Report of the Treasurer:* The Treasurer made the following report for the year 1950:

Financial Statement for the Year 1950

Balance on hand in checking account, December 31, 1949 in The Cleveland Trust Company, Cleveland, Ohio	$2,105.82	
Receipts from dues	3,877.70	
Sales of Abstracts and Proceedings	130.25	
Total credits		$6,113.77
Expenditures		
Postage, supplies and miscellaneous	115.61	
Wistar Institute, Philadelphia, Pa.		
Abstracts	485.67	
Programs	353.22	
Addressograph additions and corrections	10.46	
Printing	56.14	
Proceedings for 1949	383.38	
Proceedings for 1950	290.40	
New Orleans Meeting Expense (Aid to Local Committee)	600.00	
Legal Fees (Preparing Income Tax Exemption)	36.85	
Secretary, traveling expenses	90.00	

Bank charge for handling account	.33	
Safe Deposit Box, Central National Bank	6.00	
Enrollment		
National Society Medical Research	100.00	
National Academy of Science, Dues A.I.B.S.	660.00	
Total expenditures		3,188.06
		$2,925.71
Balance on hand December 31, 1950, and deposited in the name of The American Association of Anatomists in The Cleveland Trust Company, Cleveland, Ohio		$2,925.71
War Bond Defense Series F dated May 1942 in safe deposit box, Central National Bank, Cleveland, Ohio Value December 31, 1950		874.00
TOTAL ASSETS		$3,799.71

ANATOMICAL JOURNAL TRUST FUND

1950

Bonds on hand December 31, 1950		$17,000.00
Cash on hand Central National Bank, Cleveland, Ohio, December 31, 1949	854.30	
Interest received from bonds during 1950	562.50	1,416.80
TOTAL ASSETS		$18,416.80

2. Report of the Auditing Committee: Dr. Collings read the following report:

March 21, 1951

"We, the undersigned, appointed auditing committee, have examined the Treasurer's record of the American Association of Anatomists for 1950 and find the accounts to be in order as reported in the financial statement of 1950."

(Signed) M. RAYMOND COLLINGS, Chairman
ARCH E. COLE
H. STANLEY BENNETT

On motion made and seconded the reports of the Treasurer and of the Auditing Committee were accepted and approved.

3. *Elecetion of New Members:* The Secretary presented the following list of 37 persons elected by the Executive Committee to membership in the Association, the names in parentheses indicating the sponsors.

John Albert Arcadi, B.S., Notre Dame, 1947; M.D., Johns Hopkins University School of Medicine, 1950; House Officer, Brady Institute, Johns Hopkins Hospital. (William L. Straus, Jr. and Marion Hines.)

Pedro Belou, M.D., University of Buenos Aires, 1903; Titular Professor of Descriptive Anatomy Emeritus, Faculty of Medical Sciences of Buenos Aires. (W. F. Windle and J. F. Huber.)

Edward H. Bloch, B.S., University of Chicago, 1939; M.D., University of Tennessee, 1945; Ph.D., University of Chicago, 1949. Established Investigator of the American Heart Association, Western Reserve University School of Medicine. (Normand L. Hoerr and Arnold Lazarow.)

Henry Charles Browning, B.Sc., 1937, Ed.Dip., 1938, Bristol, England; Ph.D., Sheffield, England, 1942; Assistant Professor in Anatomy; Research Associate in Pathology, Yale University School of Medicine. (W. U. Gardner and Carroll A. Pfeiffer.)

Mary Lois Calhoun, B.S., Iowa State College, 1924; M.S., Iowa State College, 1931; D.V.M., 1939, Ph.D., 1945, Iowa State College; Professor and Head of Anatomy Department, Michigan State College. (Miriam Scott Lucas and H. L. Foust.)

Malcolm Breckenridge Carpenter, B.A., Columbia College, 1943; M.D., Long Island College of Medicine, 1947; Research Fellow in Neurology, College of Physicians and Surgeons, Columbia University. (Fred A. Mettler and Samuel R. Detwiler.)

Edward Lucas Chambers, A.B., Princeton University, 1938; M.D., New York University, 1943; Assistant Professor of Anatomy, The Johns Hopkins University School of Medicine, Department of Anatomy. (Allan L. Grafflin and William L. Straus, Jr.)

Rosette Spoerri Coidan, B.A., Washington Square College, New York University, 1942; M.Sc., 1944, Ph.D., 1947, New York University; Instructor, Department of Anatomy, State University Medical Center at New York College of Medicine. (Harry A. Charipper and Robert Chambers.)

Ivor Cornman, A.B., Oberlin, 1936; M.S., New York University, 1940; Ph.D., University of Michigan, 1949; Assistant Research Professor in Anatomy, George Washington University School of Medicine. (Warren Andrew and Ross C. MacCardle.)

E. Lloyd DuBrul, D.D.S., New York University, 1937; M.S., University of Illinois, 1949; Assistant Professor in Charges of the Division of Oral Anatomy, University of Illinois, College of Medicine. (Otto F. Kampmeier and Gerhardt von Bonin.)

Charles A. Ely, A.B., Washington and Jefferson, 1936; M.S., University of Hawaii, 1940; Ph.D., University of Wisconsin, 1948; Associate in Anatomy, College of Physicians and Surgeons, Columbia University. (S. R. Detwiler and Philip E. Smith.)

RICHARD STUDLEY FARR, S.B., University of Chicago, 1945; M.D., University of Chicago, 1946; Lieutenant, U. S. Naval Medical Corps, National Naval Medical Center, Naval Medical Research Institute, Bethesda, Maryland. (Peter P. H. De Bruyn and William L. Doyle.)

WESTON D. GARDNER, B.S., Pennsylvania State College, 1938; M.D., University of Pittsburgh School of Medicine, 1942; Instructor in Anatomy, Marquette University School of Medicine. (Theodore H. Bast and Walter Zeit.)

WILLIAM A. GEOHEGAN, E.E., Cornell University, 1929; M.D., Cornell University, 1942; Assistant Professor, Department of Anatomy, Cornell University Medical College. (Joseph C. Hinsey and John MacLeod.)

BARBARA SAMSON GRANGER, A.B., Mount Holyoke College, 1935; A.M., Mount Holyoke College, 1937; Ph.D., Cornell University, 1943; Assistant Professor of Anatomy, University of Southern California. (Robert B. Barden and Paul R. Patek.)

MERVYN GILBERT HARDINGE, B.S., Pacific Union College, 1939; M.D., College of Medical Evangelists, 1942; M.P.H., Harvard School of Public Helath, 1949; Assistant Professor of Anatomy, College of Medical Evangelists. (E. Harold Shryock and Laurence M. Ashley.)

FRANZ X. HAUSBERGER, M.D., University of Munich, Germany, 1935; Assistant Professor of Anatomy, The Jefferson Medical College. (George A. Bennett and A. J. Ramsay.)

JOHN EDWARD HEALEY, JR., B.S., St. Joseph's College, 1943; M.D., Jefferson Medical College, 1948; Associate in Anatomy, Daniel Baugh Institute of Anatomy, Jefferson Medical College. (Nicholas A. Michels and Andrew J. Ramsay.)

MINNIE HELLER, B.S., 1944; Ph.D., 1950, University of Chicago; Research Assistant, University of Chicago. (William Bloom and Peter P. H. De Bruyn.)

JOHN D. IFFT, A.B., Whitman College, 1935; M.A., University of California at Los Angeles, 1937; Ph.D., Yale University, 1941; Assistant Professor of Anatomy, Boston University School of Medicine. (Arthur M. Lassek and Wiliam C. Barrett, Jr.)

EDGAR P. JAYNE, B.A., University of Kentucky, 1936; M.S., University of Kentucky, 1940; Ph.D., Washington University, 1950; Assistant Professor of Anatomy, The George Washington University Medical School. (Warren Andrew and Ross C. MacCardle.)

DOROTHY DOLE JOHNSON, A.B., Bates, 1941; M.A., Vassar, 1943; Ph.D., Columbia University, 1949; Instructor, Department of Anatomy, College of Physicians and Surgeons, Columbia University. (S. R. Detwiler and Philip E. Smith.)

VIRGIL SHIELDS LEQUIRE, B.A., Maryville College, 1943; M.D., Vanderbilt Medical School, 1946; Assistant Professor in Anatomy, Vanderbilt University Medical School. (Sam L. Clark and James W. Ward.)

JOHN FRANCIS LHOTKA, JR., B.A., Montana State College, 1942; M.S., 1948, B.Med., 1950, Northwestern University; Interne, Minneapolis General Hospital. (H. A. Davenport and L. B. Arey.)

EDWARD WALTON LOWRANCE, A.B., University of Utah, 1930; M.A., University of Utah, 1932; Ph.D., Stanford University, 1937; Associate Professor of Anatomy, University of Missouri School of Medicine. (M. D. Overholser and E. D. Bueker.)

FERDINANDO MORIN, B.A., Liceo Tito Livio, Padua, 1932; M.D., University of Padua, 1937; Ph.D., University of Torino, 1943; Instructor in Anatomy, Wayne University College of Medicine. (Ernest Gardner and John D. Green.)

RAY JANNEY NICHOLS, A.B., Mississippi College, 1926; Ph.D., University of Illinois, 1934; Professor of Microscopic Anatomy, University of Mississippi School of Medicine. (Ira D. Hogg and D. S. Pankratz.)

MARLOW WILLIAM OLSEN, B.S., Iowa State College, 1929; M.S., 1930, Ph.D., 1941, University of Maryland; Physiologist, Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Maryland. (Mary Juhn and Mary E. Rawles.)

RONAN O'RAHILLY, M.B., B.Ch., B.A.O., National University of Ireland, 1943; Ainsworth Scholar in Anatomy, National University of Ireland; Visiting Associate Professor of Anatomy, Wayne University. (Ernest Gardner and F. Gaynor Evans.)

HERBERT EUGENE PEDERSEN, A.B., 1934, M.D., 1941, University of Michigan; Instructor in Surgery, Wayne University College of Medicine. (Ernest Gardner and F. Gaynor Evans.)

DONALD SCOTT, JR., B.S., Harvard, 1930; Ph.D., University of London, 1939; Assistant Professor Anatomy, University of Pennsylvania, School of Medicine. (William F. Windle and Roy G. Williams.)

SOTIRIOS JOHN TSOURAS, M.D., University of Athens, 1938; Dr. of Anatomy, University of Athens, Greece, 1943; Instructor, Department of Anatomy, University of Michigan. (Bradley M. Patten and Russell T. Woodburne.)

JAMES WELLINGTON WARD, A.B., 1929, M.A., 1932, University of Alabama; M.S., Mississippi State College, 1931; M.S., University of Minnesota, 1945; Ph.D., University of Mississippi, 1950; Associate Professor, Department of Anatomy, University of Mississippi, School of Medicine. (Ira D. Hogg and D. S. Pankratz).

JOHN RENSSELAER WHITTIER, B.A., Harvard, 1939; M.D., College of Physicians and Surgeons, New York City, 1943; Research Assistant, Department of Neurology, Columbia University. (Fred A. Mettler and S. R. Detwiler.)

HARRY H. WILCOX, B.S., 1939, M.S., 1940, Ph.D., 1948, University of Michigan; Associate in Anatomy, University of Pennsylvania, School of Medicine. (William F. Windle and Roy G. Williams.)

ROBERT EUGENE YOSS, M.D., University of Tennessee, 1948; Instructor in Anatomy, University of Michigan Medical School. (Elizabeth C. Crosby and Edward W. Lauer.)

SIGFRID ZITZLSPERGER, Aerztliches Staatsexamen, Munich, 1938; Dr. Med., Berlin, 1941; Dr. Med. Habil., Kiel, 1943; Instructor of Anatomy, University of Tennessee. (Frank Harrison and Normand L. Hoerr.)

On motion made, seconded, and voted, the Secretary was instructed to cast a unanimous ballot ratifying the election of these new members.

4. *Report of the Motion Picture Committee:* Professor Carl Speidel read the following report.

"At the International Congress of Anatomy, held in Oxford in July, 1950, a resolution was passed envisaging the setting-up of an International Committee for Anatomical Films. Dr. H. Kuhn, of Paris, appointed by the President of the Congress, Prof. Le Gros Clark, to carry out the necessary preliminary steps, has sent to Secretary Hoerr a tentative plan which, if approved, shall form the basis for an organization meeting, to be called later. Dr. Hoerr has referred the matter to this Committee for recommendation. We recommend that the Association endorse the general program laid out by Dr. Kuhn. We suggest that Dr. Kuhn be informed that most of the details included in his tentative plan have been incorporated, on a national scale, starting in 1940, in the motion picture activities of The Wistar Institute of Anatomy and Biology, in Philadelphia. Dr. E. J. Farris, Executive Director of The Wistar Institute, who has met with this Committee, informs the Committee that he can assure the complete cooperation of The Wistar Institute in the carrying out of the International scheme. He will gladly supply Dr. Kuhn with information as to the details of the Wistar plan, and would be able to meet with him personally, in Paris, in the week of June 13 to 20, 1951. We recommend that Dr. Farris be appointed as a special representative of the American Association of Anatomists, in the matter.

Regarding local activities, we recommend that The Wistar Institute, which has prepared a revised catalog of films for publication in The Anatomical Record, be requested to publish this catalog as a part of the Proceedings of the American Association of Anatomists, in order that each member may have an easily available copy. We repeat (with slight modifications) a statement included in last year's report:

'In 1940 The Wistar Institute of Anatomy and Biology, under Dr. E. J. Farris, created, at The Wistar Institute, a depository for motion picture films of anatomic and biologic interest. The scheme has provided a central depository and clearing-house for biologic motion picture films. (Over 60 films have been endorsed.) There are many other valuable motion picture films, of both teaching and research interest, that have not yet been submitted to The Wistar Institute. This Committee suggests that producers who possess such reels, and would like to make them available to others, send them to Dr. E. J. Farris, The Wistar Institute, Woodland Ave. and 36th St., Philadelphia 4, Pa., for consideration, and, if approved, for listing, along with other approved films, in The Anatomical Record. For style of listing consult The Anatomical Record, vol. 101, at page 132, 1948. For details, write to Dr. Farris.

While the main purpose of the original plan was to provide a central location where films would be available to *research* workers, in practice it has been found that many of the listed films are also of value, and have been widely used, in the *teaching* of Anatomy. In response to the requests of members of the Association of Anatomists, Dr. Farris will gladly list *available* films that are chiefly of teaching interest, either as films endorsed by The Wistar Institute, or, in supplementary categories, as (1) films endorsed by the Motion Picture Committee of the Anatomists, or as (2) films reported to, or known by the Committee to be useful anatomic teaching films. Also, The Wistar Institute would appreciate, as in the past, the deposition with them of a copy of the film, plus two copies of a descriptive script. It is urged that members not only submit films for review, but that they also send to Dr. Farris, information regarding films — *available* for loan, rent or purchase — which they have found useful in teaching.' "

(Signed) ELIOT R. CLARK,
for the Committee.

On motion made and seconded, the report of the Motion Picture Committee was approved, and the recommendations therein adopted. Dr. Speidel also reported that Dr. Markee was sending a questionnaire on teaching films to the entire membership, and would make a report when the results were tabulated.

5. *Report of the committee appointed to consider the question of establishing an anatomical review journal:* Dr. Cummins read the following report.

"The undersigned committee, appointed last year to consider the advisability of establishing a journal devoted to anatomical reviews, recommends that the Association take no steps in that direction at this time.

The recommendation is based chiefly upon the following considerations. (1) Publication of the *Ergebnisse der Anatomie und Entwicklungsgeschichte* is being resumed, under the editorship of Professor Curt Elze who states that he will welcome contributions from American workers. (2) Financial support of a new review journal is a difficult problem, even under circumstances more favorable than those presently existing.

Dr. George Clark had kindly placed at the disposal of the committee the complete file on his study (1949–1950) of reactions of American anatomists to various questions concerning a review journal. All his material has been carefully studied, and it should be noted that the prevailing opinion at that time favored a review journal. Dr. Clark's summary of the poll is appended; the original questionnaires and related correspondence are filed, with his consent, in the records of the Association.

The committee further recommends that the Association take formal action on Professor Elze's invitation (extended in letter of December 22, 1950) to nominate an American member on the editorial board of the *Ergebnisse*."

Respectfully submitted,
(Signed) Harold Cummins, Chairman
Warren O. Nelson
Ernst Scharrer

Dr. George Clark's tabulation of the returns from his questionnaire, 1949–50

1. How long have you been a member of the American Association of Anatomists? years.*

2. Do you consider that presently available outlets for review articles primarily anatomical in character adequate? Yes 36, No 142.

3. Some reviews are essentially annotated bibliographies while others are of a more critical type. Are either of these adequately covered? Bibliographic Yes 50, No 99. Critical Yes 21, No 136.

4. Should the writing of review articles be encouraged? Yes 162, No 14.

5. Would a journal devoted exclusively to reviews primarily anatomical in character be advantageous? Yes 139, No 37.

6. Which would be preferable — a journal appearing at regular intervals throughout the year 72 or a yearly volume similar to the Annual Reveiw Series 80.

7. Would you personally subscribe to such a journal? Yes 92, No 65.

8. Should the American Association of Anatomists participate in the promotion of such a journal? Yes 146, No 27.

9. If such a journal were established should reviews be solicited? Yes 155, No 9.

10. If you received such a request (assuming the field to be covered was within your specific interest) would you be willing to comply with it? Yes 153, No 20.

11. Remarks.

Total tabulated to date 188.

* The membership periods, as associated with the answers to question number 2, are:

	Yes	*No*	*Percentage "No"*
0–5	2	29	94
6–10	10	44	81
11–20	10	48	83
Over 20 years	14	21	60

The report of the special committee was accepted, and its recommendations were approved. On nomination of Dr. Cummins, Ernst Scharrer was elected to be the American member of the editorial board of the *Ergebnisse.*

6. Report on Anatomische Nachrichten: Dr. Hans Elias read the following report.

"In 1949, it became increasingly difficult to mail printed matters and manuscripts between the Eastern and Western Zones of Germany. The three anatomical periodicals that appear in Eastern Germany became practically inaccessible to the West.

Dr. H. von Eggeling who had been editing the Anatomische Anzeiger for many years retired from his chair in Berlin and moved to the West, to Neustadt am Ruebenberge (Hannover). H. Stieve took over the editorship of the Anatomische Anzeiger. The occupational division of Germany, however, had made it impossible for the Anatomische Anzeiger to continue as the official organ of the Anatomische Gesellschaft.

Von Eggeling, who celebrated his 80th birthday last year, established a new Journal, the ANATOMISCHE NACHRICHTEN which took over the functions of the Anatomische Anzeiger.

At present three general anatomical Journals appear in the East:

ANATOMISCHER ANZEIGER

MORPHOLOGISCHES JAHRBUCH

ZEITSCHRIFT FUR MIKROSKOPISCH-ANATOMISCHE FORSCHUNG, all three under the one-man editorship of H. Stieve.

In the West (including Austria) the following Journals appear:

ZEITSCHR. FUR ANATOMIE UND ENTWICKLUNGSGESCHICHTE

ZEITSCHR. FUR ZELLFORSCHUNG UND MIKROSKOPISCHE ANATOMIE

ANATOMISCHE NACHRICHTEN

in addition: the

ZEITSCHR. FUR ENTWICKLUNGS-MECHANIK which is highly specialized.

The continuance of the ANATOMISCHE NACHRICHTEN is endangered for scarcity of subscriptions.

The ANATOMISCHE NACHRICHTEN contains:

1. Short, original research papers, very much like those in the Anat. Record.
2. Progress resports on Anatomy in General and on its teaching, reports on meetings and a news service.
3. Papers read at the anatomists meetings held in Germany. These are longer than our own abstracts, some of them even illustrated.
4. Extremely valuable biographical articles (necorlogues).
5. A book review.
6. An international bibliography, i.e. classified titles of publications, under the editorship of Prinzhorn.

It may be mentioned that the Anat. Nachr. are open also to American authors.

The reason for the impending discontinuance of the Anat. Nachr. is the fact that it has only 100 subscribers. And the reason for this, in turn, is that very few know of its existence.

This Journal does not only perform an important service to the progress and to the history of anatomy; but it is the personal opinion of the reporter that any opportunity given to the Germans to make peaceful contributions to the intellectual progress of mankind will distract them to a certain extent from military ambitions.

What the Journal needs are subscriptions. It is therefore suggested that the membership of the Association get subscriptions immediately upon return to the home universities. It would be desirable that each departmental library, and wherever possible also the central university library as well as the local public and medical libraries be urged to subscribe to the ANATOMISCHE NACHRICHTEN, published by Piscator Verlag, Stuttgart, Germany.

The time set for discontinuance is the end of May. So, subscriptions should be made well in advance of that date."

(Signed) Hans Elias
The Chicago Medical School

7. *Membership in the American Institute of Biological Sciences:* On recommendation of the Executive Committee, and on motion made and seconded, it was voted that the Association continue its membership in the A.I.B.S. Dr. Louis Flex-

ner was re-elected for a three year term as our representative to the Governing Board of the A.I.B.S. His report for the year 1950 is published later in this report.

8. National Society for Medical Research: On recommendation of the Executive Committee, it was voted that $100 be paid to the National Society for Medical Research, to indicate our approval of the work this Society is doing. A recent report is as follows:

"Passage of a 'pound law' bill was enacted by the South Dakota Legislature and signed by the Governor on February 15th. The law becomes effective on July 1st.

The bill provides that animals needed for medical experimental purposes may be obtained under license issued by the State Department of Health from establishments maintained by municipalities for the impounding, care and disposal of animals seized by lawful authority.

Institutions that may apply for such license are schools and colleges of agriculture, veterinary medicine, medicine, pharmacy, dentistry or other educational or scientific institution properly concerned with the investigation of, or instruction concerning the structure of functions of living organisms, the cause, prevention, control or cure of diseases or abnormal conditions of human beings or animals.

The State Department of Health is empowered to adopt rules and regulations necessary to insure the humane use of animals in such experimentation with the right to inspect or investigate institutions, licenses or applying for license to secure experimental animals.

The South Dakota action brings to a total of three, states that have recently passed similar legislation. Thirty-one communities also have effected policies concerning the disposal of unclaimed impounded animals for medical use. Legislation is now pending in seven other states."

9. Report of Committee on Nomenclature: In Dr. Corner's absence, the Secretary gave a summary of the report, which follows in full.

"At the International Anatomical Conference held at Oxford, England in July 1950, which was attended by representatives of 36 countries, one session was devoted to a discussion on the best means of obtaining an international uniformity in anatomical nomenclature. The session was opened by Dr. Corner and was participated in by representatives of England, France, Holland and various other countries. Although there was considerable divergence of views, the burden of the discussion was definitely in favor of a moderate revision of the BNA. A resolution was proposed that an international committee, composed of representatives of the existing anatomical societies of the world, should be formed to try to reach agreement on an international nomenclature. This resolution was unanimously voted at the final plenary session of the Congress and Prof. W. E. LeGros Clark of Oxford University, president of the congress, was directed to lay the matter before UNESCO with a request for funds and intellectual support for this committee.

Prof. LeGros Clark placed the matter before the Secretary of UNESCO in October 1950. An officer of UNESCO replied on January 15, 1951 suggesting that a small working committee of about 7 members should be organized. This committee would determine the general principles to be followed, and conduct a program of revision to be submitted to the Anatomical societies concerned, with a view to discussion and acceptance at the next International Congress. Prof. LeGros Clark asked me to suggest countries which should be represented. In order not to delay action, I sent my personal suggestion which was as follows: Britain, France, Holland, Germany or Austria, Italy, a Scandinavian country to be chosen. Spain would be a good alternative for Italy. I should favor trying to get a Russian or Czech representative. I would also suggest enlarging the committee to include a representative of Latin America. I informed Prof. LeGros Clark that my action would be submitted to the Association, which could take official action about March 20, 1951. This personal action was reported to Dr. Hoerr and to the other members of the committee, all of whom approved my reply with suggestions for changes in detail. The inclusion of a Japanese or other Asiatic representative was suggested by Danforth. One member suggested specifying Uruguay among the Latin American Countries. One member doubts the possible utility of including a representative from the Soviet-controlled countries.

Your chairman reuests submission of the plan to the Association or to the Executive Committee as the officers may think best, and will appreciate any instructions the Association has to offer with regard to the correspondence now in progress leading up to the international action.

Prof. Danforth regretfully asks to be permitted to resign from the Committee. Your chairman with equal regret suggests that Prof. Danforth's resignation be accepted with the request that he will consent to serve as consultant from time to time.

GEORGE W. CORNER, M.D.
Chairman

On motion made and seconded, the report was approved. It was voted that the Committee on Anatomical Nomenclature be authorized to make suggestions, as the members see fit, to the UNESCO committee, if and when it is formed. Dr. Corner was elected as the representative of the Association to serve on the UNESCO committee. On recommendation of the Executive Committee, it was also voted that the Committee on Anatomical Nomenclature prepare a list of new terms, not covered by the BNA, to be published in The Anatomical Record as suggestions for inclusion in a new nomenclature.

10. Report on National Research Council: Dr. Marion Hines gave a delightful report on the activities of the National Research Council, which unfortunately was not recorded.

11. Place of next annual meeting: President Clark announced that the Executive Committee had voted to accept the invitation of Brown University to meet in Providence, R. I., on March 19, 20, and 21, 1952. These dates are several weeks before Easter next year, but are during the Spring vacation of Brown University, and the only time they could hold our meeting.

Prof. Wilson informs us that the University Cafeteria will probably sell us a package of five or seven meals for a fixed price. The cost should be considerably less than meals in hotels. We have already experimented with this method at the University of Wisconsin at the 1948 meeting. We shall probably be able to provide rooms for all registrants in the University dormitories and new housing project.

Although the costs for room and board will be less than usual, it will be necessary to know the exact attendance in advance. Therefore, a reservation ticket will probably have to be paid in advance of the meeting. Further details will be sent to the members about December 1.

Since, in these days, convention hotels often have to be reserved more than eighteen months in advance, the Executive Committee also voted tentatively to accept the invitation of Ohio State University to meet at Columbus, Ohio, in 1953. The dates will be April 1, 2, and 3, 1953.

12. The nominating committee for 1951, consisting of H. Stanley Bennett, James O. Foley, and Davenport Hooker, chairman, nominated Joseph Markee and Harold Mossman as members of the Executive Committee for four year terms. In the absence of other nominations, these gentlemen were declared elected by unamimous ballot.

13. Payment to local committee: On recommendation of the Executive Committee, it was voted to pay $800 to the local committee to help defray the costs of the meeting. Drs. Scott and Gardner stated that any unexpended balance would be returned to the Association. (Dr. Gardner returned $52.23, so our payment actually came to $747.77.)

14. Resolutions of Appreciation: Prof. Marion Hines proposed a vote of thanks to the local committee for all their work to make the meeting a success, and a vote to them and their wives for their gracious hospitality, making our meeting in Detroit a great privilege.

Dr. David Bassett moved a resolution of appreciation to the Parke, Davis and Co. for the gift of $1000, which made it possible for us to reduce the price of the banquet tickets, and enabled the local committee to provide better facilities for projection and for the demonstrations than would have been possible otherwise.

Both of these resolutions were adopted with enthusiasm.

15. Annual dues: The Executive Committee recommended that, with dues for United States citizens remaining at $5.00, dues for Canadian members be reduced to $4.00 and for foreign members to $2.00. The Canadian members receive less benefit than United States members from our memberships in the National Society for Medical Research and the American Institute of Biological Sciences. Foreign members ordinarily derive their main benefit of membership in receipt of the Abstract Supplement, and should not have to pay for costs which are purely national. This recommendation was voted.

16. Representatives, and members of standing committees: In the absence of any new nominations, all representatives and standing committees were continued for another year.

LATE ABSTRACTS

The following three abstracts were received too late for inclusion in the Abstract Supplement.

59a. The importance of the anconeus in lateralward abduction of the ulna during pronation. Robert J. JOHNSON and Robert D. RAY, Departments of Anatomy and Surgery, University of Washington School of Medicine. (Introduced by H. Stanley Bennett.)

Lateralward abduction of the ulna during pronation is demonstrated by double-exposure roentgenograms with the humerus fixed in position by skeletal pins fastened in a rigid frame. It is apparent that the axis about which pronation and supination will occur can be selected by varying the degree of lateralward abduction of the ulna. This mechanism prevents the hand from moving medialward by its own width during pronation and nullifies the need for compensatory abduction of the humerus. The ulna is deflected lateralward by about eight to nine degrees during the motion between extreme supination and extreme pronation. Cinefluorography confirmed the findings by double-exposure roentgenograms.

By means of a concentric needle electrode inserted into the anconeus electromyograms were made which showed a significant activity throughout the range of pronation. This confirms the finding by palpation. In contrast, electromyograms of the extensor carpi ulnaris showed no significant activity during pronation. These are the only muscles whose attachments could account for the abduction of the ulna. The electromyograms showed little activity of the anconeus during extension of the forearm.

D36a. The perivascular space of the pulmonary vessels. An anatomical study. Alfred M. TOCKER and Hiram T. LANGSTON, Department of Surgery, Wayne University College of Medicine. (Introduced by John D. Green.)

By injection of a solution of bismuth oxychloride in diodrast under pressure into the cleavage plane between the pulmonary vessel walls and the surrounding tissues at the hilum of the dog's lung, a definite perivascular space is demonstrated grossly, microscopically and roentgenographically. By injection peripherally, the existence of a continuous cleavage plane around the most distal branches and tributaries of these vessels is demonstrated. Injection centrally results in a spread of the solution across the midline with eventual demonstration of this perivascular space of the corresponding vessels of the opposite lung. In such a manner, the space surrounding the pulmonary artery and its branches far out into both lung fields is demonstrated by a single injection. In the case of the veins, the cleavage planes of both the superior and inferior pulmonary veins are continuous with each other on the same side, as well as with the space surrounding the veins in the opposite lung.

It is speculated that at the hilum this perivascular sheath represents an extension of the fibrous pericardium along the vessels. The pericardial space itself is separated from this perivascular space by the reflection of the serous pericardium.

The information acquired concerning the nature and extent of these perivascular spaces is of great practical value in performing pulmonary resection in clinical cases.

D51a. On the amount and position of the gravital reactions through the human foot, in walking. John C. KOCH, Detroit, Michigan.

Preliminary to making a mathematical analysis of the mechanical strength of the bones of the foot, the writer has designed and built an apparatus to determine the reactions through the metatarsal bones when the entire weight of the body is carried by the fore-foot. These 5 elemental longitudinal arches form a partial dome having great compressive stability, resilience and adaptability.

The apparatus consists of two structural steel angles, 18 and 54 inches long, each of which is independently supported, at the ends, by two bath-room scales. These angles are placed, parallel, side to side, and with their top surfaces lying in the same horizontal plane. The subject whose right foot is to be tested, stands near the middle of the angle-beams with the medial border of the right foot parallel to the narrow slit between the two beams, so that the great toe is to the left of the slit, the remaining portion of the foot being to the right. The subject then balances the entire body-weight on the ball of the right foot and simultaneous readings are made of the 4 scales. In succession, similar readings are made of the scales for the positions in which the first and second toes are to the left of the slit, and so on. From this data, the reactions of each of the metatarsals can be computed.

The Meeting of the Executive Committee
Tuesday, March 20

There was insufficient time at the business session to report the following items of business transacted at the Executive Committee Meeting.

1. Dr. Davenport's report as representative of the Association to the Stain Commission is as follows:

Annual Meetings:

A. The annual meeting of the Biological Stain Commission, Inc., was held in the library of the Department of Anatomy of the University of Pennsylvania, School of Medicine, March 24, 1950. It was opened by a call to order at 9:00 A.M. by President H. J. Conn.
Committee reports:

Dr. Lillie reported for the Committee on Mounting Media. The results of the Committee's work appeared in *Stain Technology:* 25, 1–12. 1950.

Dr. Davenport reported for the Committee on Rules of Acceptance of Stains for Certifications. The Rules were incorporated into the minutes of the Meeting.

Dr. Stotz reported for the Committee on Revision of Methods for Testing Stains for Certification. Two articles covering this activity have been publieshed in *Stain Technology:* 25, 57–68 and 25, 201–208, 1950.

The reports were accepted by the Commission.

Election of Trustees at Large:

Drs. Kornhauser and Windle were reelected for a term of one year.

The meeting adjourned about 10:30 A.M.

Members present were: H. J. Conn, Mary Darrow, H. A. Davenport, Edward W. Dempsey, F. H. J. Figge, Jacqueline P. Greco, E. K. Kline, S. I. Kornhauser, Alice M. Laskey, R. W. Mowry, E. H. Stotz, W. F. Windle and Conway Zirkle.

B. A meeting of the Trustees of the Biological Stain Commission followed immediately with the same attendance as listed above, with the addition of Dr. Louis Gershenfeld. The members of the Commission who were not trustees were invited guests at this meeting.

The President's Report was given by Dr. Conn, and the Treasurer's Report by Dr. Stotz. Dr. Conn reported also for the Finance Committee and for the status of Biotech Publications. The Publications have been self-supporting during the past year.

Dr. Windle reported for the Committee on Pensions and stated that sufficient funds had been made available to handle present requirements.

Dr. Gershenfeld's motion to adjust Dr. Conn's pension at the time his policy matures carried.

No reports were made by the Committee on Research Grants nor the Committee on Publications.

The present officers of the Commission were reelected for one year.

The following were elected to membership in the Commission:

Charles A. Woerner, Elizabeth Leduc, John F. Lhotka, Robert W. Porter, H. Patterson Mack and Arthur La Velle.

An appropriation of $10,000 to be used for research grants was made on motion by Dr. Stotz. This sum did not include the operational expenses incident to the testing of stains, since these were provided by a separate grant to the University of Rochester. The motion included the provision that requests for research grants be first passed upon by the Committee on Research, and if approved, submitted to the Finance Committee with power to act.

Dr. Dempsey's motion that the Finance Committee be empowered to dispose of or reinvest any securities of the Biological Stain Commission carried.

Other minor items, including a proposal to allow University Microfilms of Ann Arbor to distribute copies of *Stain Technology,* were discussed informally. The matter of microfilm was referred to the General Publications Committee.

The Trustees meeting adjourned about 12:30 P.M.

2. *Report of Regional Meeting in Milwaukee,* held at Marquette University, November, 4, 1950.

Fifty-eight people attended the Regional Meeting of Anatomists at Marquette University on November 4, 1950. Besides the local universities there were visitors from Turin, Italy and the University of Alabama.

Doctor Sullivan presided at the morning scientific session. At noon Doctor Clement A. Fox showed pictures that he took on his trip to the International Meeting of Anatomists at Oxford. Doctor Job presided at the afternoon scientific session. A group of demonstrations were on display in the laboratory and were observed and discussed by many of the group.

It seemed that most everyone enjoyed and approved of the meeting and it is anticipated that the group will meet in Chicago next fall.

DAVID S. JONES
Organizer ex-officio

The Executive Committee voted to recommend that regional meetings be encouraged. Dr. Duncan reported that the Texas anatomists are planning a regional meeting, possibly to be held this autumn. The Executive Committee voted not to print the programs of regional meetings in the Proceedings.

3. *Dr. Louis Flexner, our representative to the Governing Board of the A.I.B.S., submitted the following report:*

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES

Member Societies. The Institute now has 16 member societies, 4 affiliate societies and 3 associates. The non-duplicate memberships of the 16 member societies is 14,000 and there are 12,000 additional in the 4 affiliate societies.

Officers. F. P. Cullinan, Chairman; W. O. Fern, Vice Chairman; C. J. Hylander, Executive Secretary.

Financial Report for 1950–1951:

Estimated income		$21,000.00
Estimated expenses		
Salaries (four persons)	$16,000.00	
Travel	2,000.00	
Mailing	2,000.00	
Office supplies	1,000.00	
News Letter and Bulletin	5,000.00	
Total expenses		$26,000.00

The indicated deficit is expected to be met from new sources of income such as noted under (4) below; societies will not be asked to increase their dues. Activities of the Institute include:

1. *A.I.B.S. Placement Service.* This was inaugurated at the Ohio State meeting of fourteen biological societies. At present there are over 100 registrants, and currently a half dozen openings. Registration is free to members of A.I.B.S. member societies; $3.00 to affiliates; and $5.00 to the general non-member. An annual salary percentage of 2% (which is one-half to one-third that charged by other placement agencies) will finance this activity.

2. *The A.I.B.S. Bulletin.* This is to be a quarterly, replacing the News Letter, which will be mailed free to members of A.I.B.S. member societies and which will include current information of interest to biologists. Advertising income is expected almost to cover the costs of printing and mailing the Bulletin.

3. *A.I.B.S. and the National Scientific Register.* The development of a national roster of biologists has been an important goal for A.I.B.S.; it is now in the process of being realized. The A.I.B.S. Committee on the Roster of Biologists prepared the specialty check list included in the questionnaire and A.I.B.S. has been given the responsibility of sending the questionnaire to all qualified biologists. By July 1 it is expected that 33,000 biologists will have been reached in this way.

4. *A.I.B.S. Financial Support.* The Rockefeller Foundation has been asked for assistance so that such activities as a centralized journal publication office can be realized. At the same time industrial organizations are being approached with the purpose of increasing the number of associates. These two avenues of income should add considerable to that provided by membership society dues.

5. *Meetings.* Last year 6 A.I.B.S. societies requested A.I.B.S. to organize a joint meeting at Ohio State University; 8 non-member societies joined in the meeting. This enterprise involving 2,000 participating biologists and a $9,000 budget was carried through without

drawing upon the general funds of A.I.B.S. This year the same 6 societies are meeting under A.I.B.S. auspices at the University of Minnesota; this meeting will be self-supporting.

6. *New A.I.B.S. Affiliates.* Accepted are American Society of Limnology and Oceanography, and Society for the Study of Evolution. Application for affiliate membership has been received from the American Society of Human Genetics.

7. *Biological Abstracts.* The Office of Naval Research gave $22,000 through A.I.B.S. to expedite the 1949 and 1950 indices of Biological Abstracts.

Report of C. J. Hylander,
Executive Secretary of A.I.B.S.
as condensed by L. B. Flexner

4. *The executive committee of the Tissue Culture Association* had inquired concerning the possibility of having abstracts of papers presented at their meeting published with our Proceedings. The executive committee voted that only abstracts of papers presented at our own meeting should be published by our Association. However, the committee expressed a desire to have the program of the Tissue Culture Association Meeting announced to our members in some way. Many of our members would have attended the T.C.A. meeting if they had been informed of it.

5. *The executive committee,* on the recommendation of President Clark, elected Dr. Raymond Truex and Dr. John Huber to be our representatives at the 1951 meeting of the American Association for the Advancement of Science, to be held in Philadelphia beginning on December 26.

6. *Dr. Karl Mason's term as member of the Journal Trust Fund committee* expired this year. Since this committee has had no business to transact since the incorporation of the Association in 1946, the Executive Committee recommended that the present members of the Journal Trust Fund committee be continued indefinitely. The present Motion Picture Committee was re-elected. Dr. Davenport was re-elected as our representative to the Stain Commission.

7. *Dr. Clark brought up the question of reducing the term of the president to one year.* Dr. Hoerr brought up the question of splitting the secretary-treasurership, and the sugges-

tion that we have a program secretary in charge of the annual meeting. The Executive Committee voted to take no action on these questions at this time.

8. Membership. On March 1, 1950, the Association had 927 members. This number has been reduced during the year by eight deaths and four resignations. With the election of 37 new members, the Association now has 952 members. Ten members were added to the non-paying list, making a total of 61 members in this category.

Members Deceased. The Association has suffered very great loss through the recent deaths of the following members:

We have received the following from Dr. G. Bevelander, College of Dentistry, New York University.

MOSES DIAMOND, Professor and Chairman of the Research Committee in the Dental College at Columbia University died October 6, 1949 at the age of 55.

Dr. Diamond was educated in the public schools in New York and graduated from the New York College of Dentistry in 1914. Following his graduation, he served in the Sanitary Corps of the United States Army in 1917 during which time he was an instructor in x-ray techniques. In 1920 he was appointed lecturer at Columbia University; the following year was spent in England where he lectured at Guys Hospital. During his tenure in England Dr. Diamond also lectured before the Royal Society of Medicine on topics related to restoration and preventive dentistry.

On his return to this country in 1922 Dr. Diamond joined the staff at Columbia University again and continued his association with this institution until his death.

Dr. Diamond was active in teaching, research and in the correlation of basic sciences and their application to clinical practice. His publications include a well known text book of Dental Anatomy, a translation of a monograph in dentistry and several original research publications dealing with histology, embryology and morphology of the teeth.

Dr. Diamond was a member of several learned societies including The American Association of Anatomists; The New York Academy of Sciences; The International Association for Dental Research; The American College of Dentists; Sigma X and O.K.U.

The following was received from Dr. W. R. Ingram, College of Medicine, State University of Iowa.

Avery Eldorus Lambert was born October 31, 1873, in Waldoboro, Me., and died in Iowa City on November 16, 1950. He attended Dartmouth College, obtaining the B.S. in 1902 and the Ph.D. in 1906. After serving as instructor and professor in Biology at Framingham Normal School (1904–1910) and Middleburg College (1911–1916) he became professor of Anatomy at the University of Vermont, from where he was called to become professor and head of Anatomy at the University of Alabama. In 1925 he became professor of Histology at the State University of Iowa, a post he held until he became Professor Emeritus in 1944. Dr. Lambert's scientific contributions ranged from paleontology to gross anatomy, and he was the author of the widely used "Introduction and Guide to the Study of Histology," which has gone through several editions. Dr. Lambert was a man of diversified interests. He was active in community life, was a popular speaker for lay audiences, including radio, and was the author of a book of poems.

The following was received from Dr. O. Larsell, University of Oregon Medical School.

William Fitch Allen was born on November 11, 1876, at Oneonta, New York. He grew up in small country parsonages, his father having been a Presbyterian minister in upper New York State. Following graduation from high school he entered Cornell University, but after two years there transferred to Stanford University, from which he received the B.A. degree in 1900 and the M.A. in 1902.

At Stanford Allen came under the immediate influence of David Starr Jordan, the eminent ichthyologist, who also was president of the university, and of Charles Henry Gilbert, professor of zoology. He began a series of studies on the blood vascular and lymphatic systems of cyclostomes and fishes by injection methods which resulted in several beautifully illustrated monographs, published in the Proceedings of the Washington Academy of Sciences, in the Quarterly Journal of the Microscopical Society of London, and in the American Journal of Anatomy, from 1905 to 1913.

In 1901 he became assistant to E. P. Allis, collecting and preparing material for this investigator of the morphology of generalized fishes. From 1906 to 1910 he was associated with Jacques Loeb at the Pacific Grove Marine Laboratory, continuing his researches on the vascular systems.

When Loeb was appointed Director of The Rockefeller Institute, in 1910, Allen became instructor in comparative anatomy and embryology at the University of Illinois. The following year he went to the University of Minnesota as instructor in embryology and neuro-anatomy, working with J. B. Johnston, one of the founders of the ''American School'' of neurologists. Allen took his doctorate under Johnston's direction in 1915, his dissertation dealing with the spinal cord and medulla oblongata of cyclostomes. From this time on his research interests were centered on the nervous system.

The University of Oregon Medical School, then in process of transformation from the old type institution in which teaching was entirely in the hands of clinicians, invited Allen to become professor of anatomy. He began this service in 1916 and continued until his retirement as professor emeritus in 1946. After relief from teaching duties, however, he still came to his laboratory 7 days in the week, carrying on his research until two weeks before his death.

For many years his investigations were concerned with fiber pathways and centers of the visceral and reticular systems, but he also made important contributions to the efferent tracts of the cerebellum, the mesencephalic Vth tract, and others. His interests gradually turned toward functional aspects of the nervous system and during the last 25 years his research was largely physiological, with checks by neuroanatomical methods, most of his papers during this period having been published in physiological journals. From about 1936 on he developed techniques for testing correct conditioned responses, after lesions of various parts of the cerebral cortex or of fiber tracts in previously trained dogs. The results have been published in an important series of papers. The last series of experiments, extending over a period of two years, was completed about two months before Dr. Allen's death. The manuscript embodying the results was nearly finished when he was taken to the hospital. During the first week, while tests were being made to determine the nature of his illness, his mind was clear, and the paper, which will be published posthumously, was completed on his sickbed. He died March 11.

Dr. Allen was an amusing individual, of simple tastes and with a keen analytic mind. His activities revolved entirely around his researches, at which he worked every day in the year, rarely taking vacations. In addition to his contributions to neuroanatomy and neurophysiology, his zeal for research kas an important factor in transforming the institution in which he spent the greater part of his academic life from an unkonwn medical school into a modern institution for medical teaching and investigation.

His bibliography numbers 57 titles, including the final paper which has been submitted to one of the journals.

The following was received from Dr. William W. Greulich, Stanford University School of Medicine.

Frank Mace MacFarland, emeritus professor of histology at Stanford University, and a member of its faculty for almost sixty years, died suddenly in San Francisco on February 21st. He was attending the first meeting of the California Academy of Sciences in its new auditorium which he had been instrumental in planning.

Dr. MacFarland was born in Centralia, Illinois, on June 10, 1869, the son of Dr. Parker M. and Sara Elizabeth (Mace) MacFarland. He received his Bachelor's degree from DePauw University in 1889, his Master's from Stanford in 1893 and his doctorate from the University of Wurzburg in 1896. In Europe he studied at various centers of learning, including Freiburg, Zurich, Wurzburg and the famous biological station at Naples. His genial disposition and unusually wide scientific interests brought him a wide and intimate acquaintance with many of the most distinguished biologists of the period. In later years his reminiscences of European university life and famous personalities in Europe at the close of the last century were a delight to his younger colleagues.

In the early summer of 1892 Dr. MacFarland and his lifelong friend, the late Professor George Clinton Price came to Stanford as instructors and advanced students, less than a year after the University first opened. During his long association with the University, Dr. MacFarland contributed much to its development. He played a leading role in organizing the Hopkins Marine Biological Station at Pacific Grove, of which he was in charge from 1910 to 1913 and co-director from 1915 to 1917, and in which he maintained an active interest throughout the remainder of his life.

On August 27, 1902, he married Miss Olive Knowles Hornbrook who has devotedly assisted and participated in his extensive studies, both as a skilled technician and as an artist whose delicate water color drawings have illustrated many of his scientific publications. Long and intensive study of the nudibranchs, a large group of marine molluscs of beautiful colors and bizarre forms, brought Dr. MacFarland worldwide recognition as the greatest authority on the life and habits of these animals. At the time of his death he was bringing to completion a comprehensive monograph on the group.

In addition to membership in many other scientific organizations both in this country and abroad, Professor MacFarland was very actively associated with the California Academy of Sciences, of which he was successively, corresponding secretary, first vice-president, and for fourteen years its president. Following his retirement from teaching he also served for five years as acting director of the Museum and the Steinhart Aquarium. In 1946 he was made honorary member of the Academy, the highest honor the Academy can bestow. He was an honorary life member of the National Geographic Society, a corresponding member of the Malacological Society of London, and of numerous other learned societies.

In his death Stanford has lost another of that goodly company, its early faculty, whose labor created the University and whose productive scholarship gained for it a distinguished position among the institutions of higher learning in this country.

The following was received from Dr. Karl Mason, The University of Rochester, School of Medicine and Dentistry.

ARTHUR EMERSON WARREN was born at Everett, Massachusetts, on February 14, 1897. After receiving his B.A. degree at Acadia University in 1923, he pursued graduate study and served as Assistant in Biology at Yale (1923–1926) and Harvard (1931–1933), the intervening five years being spent in high school teaching. After receiving his Ph.D. at Harvard in 1933, he joined the Department of Biology at McMaster University, Hamilton, Ontario where he served as Assistant Professor (1933–1940), Associate Professor (1940–1944), and Professor and Chairman of the department from 1944 to the time of his death on January 6, 1951. Dr. Warren's research interests were in the fields of experimental embryology and animal ecology. He held membership in numerous scientific societies in the United States and Canada. Through his enthusiasm and organizational capacity, he contributed much to the scientific and intellectual life of the university and the community in which he lived.

The following was received from Drs. Philip E. Smith and Adolph Elwyn, Department of Anatomy, Columbia University.

OLIVER SMITH STRONG was born in Red Bank, New Jersey, December 30, 1864, and, after an extended illness, died February 22, 1951. He received the A.B. degree at Princeton University in 1886, and A.M. in 1888. He then was given a fellowship at Columbia University where he received his

Ph.D. in 1895. He immediately became associated with the College of Physicians and Surgeons of Columbia, retiring as Professor Neurology and Neurohistology, Emeritus, in 1937. Dr. Strong was on the editorial board of several neurological journals and was Honorary Editor of the Journal of Comparative Neurology at the time of his death.

In Professor Strong's undergraduate work he gained a deep interest in the structure and function of nervous tissue. His graduate work resulted in the publication of an outstanding thesis, "The Cranial Nerves of Amphibia," a pioneer contribution to the knowledge of cranial nerve components. He contributed a number of scientific and clinical papers to neurological journals (J. Comp. Neur., Arch. Neur. and Psyciatry, Bull. Neurol. Inst.), and was largely responsible for the organization of the neuroanatomical course at the College of Physicians and Surgeons, Columbia University. The chapters on the Nervous Tissues and Central Nervous System appearing in Bailey's Histology until 1936 were written by Professor Strong and were the forerunner of "A Text Book of Neurology" by Strong and Elwyn.

Dr. Strong was meticulous in his research and in his teaching. He was gracious, thoughtful of others and ever-willing to help his colleagues. His advice was sought profitably by the clinicians in the Neurological Institute of New York.

Dr. Strong was a member of a number of scientific societies including the American Association of Anatomists which he joined in 1908. He will long be remembered as a scholar and a kind and thoughtful colleague.

PROFESSOR H. K. CORNING died on February 7, 1951. During the past few years, Dr. Corning had been our oldest living member. Born in Philadelphia, he had pursued his education in German Universities, and was for many years Professor of Anatomy at Basel, Switzerland. He was the author of two widely known textbooks, one on embryology and one on topographical anatomy. After his retirement, Dr. Corning returned to his native land, and at the time of his death was living in New York City.

DR. BURTON D. MYERS was born on March 30, 1870, and died last October at the age of 80. Dr. Myers received his Ph.B. at Buchtel College in 1893, his A.M. at Cornell in 1900, received his M.D. from the University of Leipzig in 1902. After serving for one year as Instructor in Anatomy at Johns Hopkins, he went to the University of Indiana, as head of the Department of Anatomy in 1903. He served in this capacity for 37 years, becoming Professor Emeritus of Anatomy in 1940. Dr. Myers' greatest contribution was in the raising of standards in the teaching of anatomy. He spent many years in administrative work, serving as Dean of the school at Bloomington, Indiana for 13 years. Dr. Myers' research work was concerned mainly with embryology and with the structure of the optic chiasma.

NORMAND L. HOERR
Secretary of the Sixty-fourth Session of
the American Association of Anatomists

Abstract of paper presented from platform

45. On the source of birefringence in striated muscle.[1] R. DALE SMITH, Department of Anatomy, Creighton University School of Medicine.[2]

Entire exsanguinated rabbit muscles were subjected to extraction with potassium pyrophosphate solutions at 0°C. for periods ranging from a few days to several weeks. This treatment resulted in the liberation of proteins from the muscle. Such extracted muscles when examined in ordinary light showed remarkably little change in structure. Histologically the fibers appeared to be contracted; with contraction bands evident and appearing as the usual dense highly refractile areas. When the fibers were studied in polarized light they exhibited a marked reduction in birefringence. After a few days of extraction all of the fibers showed a general reduction in birefringence and at the end of a week only a faint trace of the original birefringence remained. The reduction in birefringence in the individual fibers was uniform and therefore entirely different from that seen in rigor mortis. Muscles held at 0° in Ringer-Locke solution for similar periods of time showed no marked changes in birefringence. Since the extraction procedure removes proteins of sufficient dimensions to show flow birefringence and the extracted muscle shows considerable loss of its birefringence it might be presumed that these proteins are responsible for at least part of the birefringence of the intact muscle. It is also indicated that the striations of muscle as seen in ordinary light are not produced by the birefringent material.

---

[1] Due to a number of errors in the previous abstract, we are reprinting herewith a corrected version.

[2] Work done at University of Maryland Medical School and aided by a grant from the Bressler Alumni Research Fund.

AMERICAN ASSOCIATION OF ANATOMISTS CONSTITUTION

(AS AMENDED APRIL 22, 1948)

Article I

Section 1. The name of the society shall be "The American Association of Anatomists."

Sec. 2. The purpose of the Association shall be the advancement of anatomical science.

Article II

Section 1. The officers of the Association shall consist of a President, a First Vice-President, a Second Vice-President, and a Secretary who shall also act as Treasurer. The President and the two Vice-Presidents shall be elected for two years, the Secretary for four years. In case of absence of the President and one or both Vice-Presidents, the members of the Executive Committeee, in order of seniority, shall preside. The election of all the officers shall be by ballot at the annual meeting of the Association and their official term shall commence with the close of the annual meeting.

Sec. 2. At the annual meeting next preceding an election, the President shall name a nominating committee of three members. This committee shall make its nominations to the Secretary not less than two months before the annual meeting at which the election is to take place. It shall be the duty of the Secretary to mail the list to all members of the Association at least one month before the annual meeting. Additional names for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

As amended at the 31st Session, 1914.

Article III

The management of the affairs of the Association shall be delegated to an Executive Committee, consisting of twelve members, including the officers. Two members of the Executive Committee shall be elected annually, and, so far as possible, election of members of the Executive Committee shall be in proportion to the geographical distribution of members. So long as the Association is incorporated in the State of New York, at least one of the officers or one member of the Executive Committee shall be a legal resident of that State.* Five shall constitute a quorum of the Executive Committee.

**Adopted as an amendment at the 61st Session, April 22, 1948.*

Article IV

The Association shall meet at least annually, the time and place to be determined by the Executive Committee. The annual meeting for the election of officers shall be the meeting of convocation week, or in case this is not held, the first meeting after the new year.

Article V

Section 1. Candidates for membership must be persons engaged in the investigation of anatomical or cognate sciences, and shall be proposed in writing to the

Executive Committee by two members, who shall accompany the recommendation by a list of the candidate's publications, together with references. Their election by the Executive Committee, to be effective, shall be ratified by the Association in open meeting.

(Candidates for membership must have received the doctor's degree [M.D. or Ph.D.] or have had equivalent experience and training, and have either published at least one independent, definitive paper — or have, on reliable testimony, been chiefly responsible for one or more of several substantial papers of joint authorship — upon anatomical or cognate subjects. *Adopted as a recommendation at the 58th Session, 1942, but never formally adopted as an amendment.)*

Sec. 2. Honorary members may be elected from those who have distinguished themselves in anatomical research. Nominations by the Executive Committee must must be unanimous and their proposal with a reason for recommendation shall be presented to the Association at an annual meeting, a three-fourths vote of members present being necessary for an election. *(The death of Prof. A. Nicholas in 1940 left the Association with no honorary members. Action on discontinuing this section has been deferred since 1933.)*

ARTICLE VI

Section 1. The annual dues of the Association shall be determined at each annual meeting for the ensuing year. A member in arrears for dues for two years shall be dropped by the Secretary at the next meeing of the Association, but may be reinstated at the discretion of the Executive Committee on payment of arrears.

Adopted at the 39th Session, 1923.

Sec. 2. Any member who has paid the annual dues for thirty years, or who has attained the age of sixty-five years, or who has retired because of illness, may be relieved of the payment of the annual dues at the discretion of the Executive Committee.

Adopted as an amendment at the 56th Session, March 31, 1940.

ARTICLE VII

Section 1. Twenty members shall constitute a quorum for the transaction of business.

Sec. 2. Any change in the constitution of the Association must be presented in writing at one annual meeting in order to receive consideration and be acted upon at the next annual meeting; due notice of the proposed change to be sent to each member at least one month in advance of the meeting at which such action is to be taken.

Sec. 3. The ruling of the Chairman shall be in accordance with "Robert's Rules of Order."

ORDERS

The following orders have been adopted by this Association:

1. Newly elected members must qualify by payment of dues for one year within thirty days after election.

Adopted at the 24th Session, 1909.

2. The maximum limit of time for the reading of papers shall be twelve minutes.

Adopted in 1941.

3. The Secretary and Treasurer shall be allowed his traveling expenses and the sum of $10 toward the payment of his hotel bill, at each session of the Association.

Adopted at the 24th Session, 1909.

4. The Executive Committee shall nominate each year, for election by the Association at the Annual Meeting, two members to serve as representatives to the Council of the American Association for the Advancement of Science at the meetings of the Council during the same year.

Adopted at the 48th Session, 1932.

5. The Executive Committee shall nominate from time to time, for election by the Association, two members to serve as representatives to the National Research Council; one to the Medical Division and the other to the Biological Division respectively, their terms of office to be 3 years.

Modified by resolution passed at the 60th Session, 1947.

6. The Executive Committee shall nominate each year, for election by the Association, one member to serve as representative to the Biological Stain Commission, the same person to be reappointed from year to year as the Association sees fit.

(Same as original order adopted at the 48th Session, 1932, except that the name of the Biological Stain Commission was originally the Committee for the Standardization of Biological Stains.)

7. The Executive Committee shall nominate annually, for election by the Association, a Motion Picture Committee of four members to cooperate with The Wistar Institute over details of the Institute's plans for housing and making available scientific motion pictures of interest to anatomists and biologists.

Adopted at the 58th Session, April 2, 1942.

(Three members changed to four members at the 62nd Session, 1949.)

8. The President shall nominate, for election by the Association, a Committee on The Anatomical Journal Trust (Minot Memorial Fund), consisting of 3 members, with terms of appointment so arranged that one member shall retire at each annual meeting; subject to reappointment if the Association approves. This committee shall have the authority to expand the income of the Fund, and the Secretary-Treasurer shall make payments against the income as directed by the Committee.

Adopted at the 60th Session, April 4, 1947.

The two following orders are proposed by the Secretary for adoption:

9. As long as the Association continues membership in the American Institute of Biological Sciences, the Executive Committee shall nominate from time to time, for election by the Association, one member to serve as representative on the governing board of the American Institute of Biological Sciences; his term of office to be three years.

(Order no. 5 originally contained provision for a representative to the Union of Biological Societies. With the dissolution of this organization, and the formation of the A.I.B.S., the above order has been in force, through a resolution passed at the 61st Session, 1948. It has not, however, been formally adopted as an order.)

10. The Executive Committee shall nominate each year, for election by the Association at the Annual Meeting, a committee on Anatomical Nomenclature consisting of a chairman and not more than six other members of the Association.

(Although a Committee on Anatomical Nomenclature was appointed at the 2nd Session in 1889, thus having been the first standing committee, no record of an order or resolution could be found in the Proceedings.)

Resolutions

The following resolutions are still in force:

1. Resolved, that the Secretary-Treasurer shall accept, on behalf of the Association, from the Trustee of the Anatomical Journal Trust Fund (Minot Memorial Fund) the funds of the trust. It is the intention of the Association to hold the corpus of this fund intact, as a safeguard against possible emergencies in the publication of anatomical journals in which the Association may be interested; and to employ the income as may seem advisable and proper, in view of the Association's status as a non-profit corporation, to promote the publication and diffusion of anatomical literature. *(Adopted at 60th Session, 1947.)*

2. Resolved, that the Association become a member of the National Society for Medical Research. The society is established for the purpose of developing, through organized effort, a better public understanding and appreciation of the methods and the principles of scientific investigation in the fields of Biology, Medicine, Dentistry, Pharmacy, and Veterinary Medicine. *(Adopted at 60th Session, 1947.)*

3. Resolved, that motion picture reels, in order to be included in the annual program of the Association, must be submitted to a committee in advance of the meeting. A preview by the committee is required to avoid serious embarrassment to local medical authorities through the activities of certain antagonistic groups. *(Adopted at 58th Session, 1942.)*

4. Resolved, that the Association nominate to The Wistar Press, editors of The Journal of Anatomy, The Anatomical Record, and The Journal of Comparative Neurology. *(Adopted in part, at the 37th Session, 1921, at least this is the only record we can find in the Proceedings.)*

5. Resolved, that the Association pay sufficient money to defray costs of meetings, so as not to be a burden on the Local Committee on Arrangements. *(Adopted at 59th Session, 1946.)*

6. Resolved, that members not intending to be present at a meeting should send their abstracts as to be read by title only. The abstracts of such communications are to be published in connection with the Proceedings of the Meeting. *(Adopted at 38th Session, 1922.)*

7. Resolved, that the Association of Anatomists join the American Institute of Biological Sciences as a full member. The purposes of the Institute are the advancement of biological sciences and their application to human welfare. *(Adopted at 61st Session, 1948.)*

AMERICAN ASSOCIATION OF ANATOMISTS

OFFICERS AND LIST OF MEMBERS

Officers

President	SAM L. CLARK
First Vice-President	GORDON SCOTT
Second Vice-President	J. C. B. GRANT
Secretary-Treasurer	NORMAND L. HOERR

Executive Committee

For term expiring 1952	W. R. INGRAM, W. U. GARDNER
For term expiring 1953	JAMES W. PAPEZ, LOUIS B. FLEXNER
For term expiring 1954	DONALD DUNCAN, MIRIAM SIMPSON
For term expiring 1955	J. E. MARKEE, HARLAND MOSSMAN

Delegates to the Council of A.A.A.S. for 1951

Raymond C. Truex
John Franklin Huber

Representatives to the National Research Council

Marion Hines
George W. Corner

Member of the Biological Stain Commission

Harold A. Davenport

Representative on the Board of Directors of the A.I.B.S.

Louis B. Flexner

Committee on Nominations for 1952

A. H. Davenport, Chairman

Edward W. Dempsey
Philip B. Armstrong

Committee on Motion Pictures

Eliot Clark, Chairman

Warren H. Lewis
Carl Speidel
Joseph Markee

Committee on Journal Trust Fund

Phillip E. Smith, Chairman

Bradley Patten
Karl E. Mason

Committee on Anatomical Nomenclature

George W. Corner, Chairman

Edward A. Boyden
Sam L. Clark
C. H. Danforth
W. W. Greulich

MEMBERS

Abercrombie, W. Fulton, A.B., Sc.M., Ph.D., Technical Editor, U.S. Public Health Service, *204 Geneva St., Decatur, Ga.*

Aberle, (mrs.) Sophie D., Ph.D., M.D., *National Research Council, 2101 Constitution Ave., Washington, D. C.*

Adams, A. Elizabeth, A.B., A.M., Ph.D., Professor of Zoology, *Mount Holyoke College, South Hadley, Mass.*

Adams, L. A., A.B., A.M., Ph.D., Professor of Zoology, Curator of Museum of Natural History, University of Illinois, *401 Vermont St., Urbana, Ill.*

Adamstone, F. B., B.A., M.A., Ph.D., Professor and Head of Department of Zoology, *345 Natural History Bldg., University of Illinois, Urbana, Ill.*

Addison, William Henry Fitzgerald, B.A., M.D., Professor Emeritus of Normal Histology and Embryology, *School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*

Adelmann, Howard B., B.A., M.A., Ph.D., Professor of Histology and Embryology and Chairman, Department of Zoology, *Cornell University, Ithaca, N. Y.*

Ades, Harlow W., B.S., M.S., Ph.D., Professor of Anatomy, Chairman, Department of Anatomy, *School of Medicine, Emory University, Emory, Ga.*

Albert, A. A., M.A., Ph.D., M.D., Head of Endocrinology Laboratory, Mayo Clinic; Ass't. Prof. Physiology, Mayo Foundation, University of Minnesota, *102–110 Second Ave., S.W. Rochester, Minn.*

Albert, Samuel, B.A., M.D.C.M., Ph.D., Instructor in Pathology, Wayne University Medical School, Detroit, Mich. Staff Member, Detroit Inst. Cancer Research, Detroit Institute of Cancer Research, *4811 John R. St., Detroit, Mich.*

Alden, Roland H., B.A., Ph.D., Professor of Anatomy and Head, Department of Microscopic Anatomy, *University of Tennessee College of Medicine, Memphis 3, Tenn.*

Alexander, William F., B.S., M.S., Ph.D., Associate Professor of Anatomy, *School of Medicine, St. Louis University, 1402 S. Grand Ave., St. Louis 4, Mo.*

Algire, Glenn H., M.D., Senior Surgeon, U.S.P.H.S., *National Cancer Institute, Bethesda, Md.*

Allen, Bennet Mills, Ph.B., Ph.D., D.S. (honorary), Professor of Zoology, University of California at Los Angeles, *147 N. Bowling Green Way, Los Angeles 24, Calif.*

Allen, Ezra, A.M., Ph.D., Retired. Visiting Professor of Biology, J. B. Stetson University, *223 W. Minnesota Ave., De Land, Fla.*

Allen, Lane, Ph.D., M.D., Professor of Anatomy, *University of Georgia, School of Medicine, Augusta, Ga.*

Allen, Willard M., B.S., M.S., M.D., *Washington University, School of Medicine, St. Louis, Mo.*

Altschul, Rudolf, M.D., Professor in Histology and Neurology, *University of Saskatchewan, Saskatoon, Canada.*

AMPIL, DOMINGO G., M.D., Instructor in Anatomy, *College of Medicine, University of the Philippines, Manila, Philippines.*

ANDERSEN, DOROTHY H., Associate in Pathology, Columbia University; Assistant Pathologist, *Babies' Hospital, 167th St. and Broadway, New York City.*

ANDREW, WARREN, M.S., M.D., Ph.D., Professor of Anatomy and Chairman, *Department of Anatomy, George Washington University, Washington, D. C.*

ANDREWS, FREDERICK N., B.S., M.S., Ph.D., Professor of Animal Husbandry, *Department of Animal Husbandry, Purdue University, Lafayette, Ind.*

ANGEL, J. LAWRENCE, A.B., Ph.D., Associate in Anatomy and Physical Anthropology, *Jefferson Medical College, Daniel Baugh Institute of Anatomy, 307 S. Eleventh St., Philadelphia, Pa.*

ANGULO Y GONZALEZ, ARMANDO W., B.L., B.S., A.B., A.M., Ph.D., Associate Professor of Anatomy, *Hahnemann Medical College and Hospital, Philadelphia, Pa.*

ANSON, BARRY JOSEPH, A.B., A.M., Ph.D., Professor of Anatomy, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*

APGAR, BESSIE D., Ph.D., *Elizabethtown College, Elizabethtown, Pa.*

APGAR, CHARLES S., JR., Ph.D., *Elizabethtown College, Elizabethtown, Pa.*

APPLEBAUM, EDMUND, D.D.S., Assistant Professor of Oral Anatomy, Columbia University, *College of Physicians and Surgeons, 630 West 168th Street, New York 32, N. Y.*

APPLEBY, J. I., A.B., M.B., A.M., M.D., Physician and Surgeon, Company Surgeon, N. Y. C. & St. L. R. R. Co., *105 1/2 East Main St., Bellevue, O.*

ARCADI, JOHN ALBERT, B.S., M.D., Research Associate and House Officer, *Brady Institute, Johns Hopkins Hospital, Baltimore 5, Md.*

AREY, LESLIE B., Ph.D., Sc.D., LL.D., (Exec. Com. '30–'34, Second Vice-Pres. '42–'46), Robert Laughlin Rea Professor of Anatomy, and Chairman of the Department of Anatomy, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*

ARMSTRONG, PHILIP BROWNELL, B.S., M.D., Professor of Anatomy, *State University of New York, Medical Center at Syracuse, Syracuse 10, New York.*

ASDELL, SYDNEY ARTHUR, M.A., Ph.D., Professor of Animal Physiology, State College of Agriculture, *Cornell University, Ithaca, N. Y.*

ASHLEY, LAURENCE M., B.S., A.M., Ph.D., Professor of Zoology, Department of Biology, *Walla Walla College, College Place, Wash.*

ASLING, C. WILLET, A.B., M.A., M.D., Ph.D., Assistant Professor of Anatomy, Division of Anatomy, *University of California, Berkeley 4, Calif.*

ATKINSON, WILLIAM B., B.S., M.S., Ph.D., Associate Professor of Anatomy, *University of Cincinnati College of Medicine, Cincinnati 19, Ohio.*

ATTERBURY, RUTH RAND, A.M., Ph.D., *San Marcos, California.*

AUER, JOSEPH, M.D., D.M.Sc., Professor of Anatomy, University of Ottawa, *20 Fisher Ave., Ottawa, Canada.*

BACON, ROBERT LEWIS, B.S., M.S., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, Stanford University, California.*

BADERTSCHER, JACOB A., Ph.B., Ph.M., Ph.D., Professor Emeritus of Anatomy, Indiana University School of Medicine, *312 South Fess Ave., Bloomington, Ind.*

BAGLEY, CHARLES, JR., M.D., Professor of Neurosurgery, University of Maryland Medical School; Associate in Experimental Neurology, Johns Hopkins University Medical School, *The Latrobe Apts., Baltimore, Md.*

BAILEY, PERCIVAL, M.D., Ph.D., Professor of Neurology and Neurological Surgery, *University of Illinois, 912 South Wood St., Chicago, Ill.*

BAILLIFF, RALPH N., B.A., M.A., Ph.D., Associate Professor of Anatomy, *Tulane University, Department of Anatomy, New Orleans 18, La.*

BAITSELL, GEORGE ALFRED, B.S., M.A., Ph.D., LL.D., Colgate, Professor of Biology, Yale University, *Osborn Zoological Laboratory, New Haven, Conn.*

BAKER, BURTON LOWELL, M.S., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, University of Michigan Medical School, Ann Arbor, Mich.*

BAKER, DAN DYSART, A.B., M.A., M.D., Orthopaedic Surgery, *15th floor, Hibernia Bank Bldg., New Orleans 12, La.*

BAKER, R. C., A.B., M.A., Ph.D., Professor of Anatomy, *Department of Anatomy, Hamilton Hall, Ohio State University, Columbus, O.*

BALDWIN, WESLEY MANNING, A.B., A.M., M.D., *Box 441, York Beach, Maine.*

BARDEN, ROBERT BRADSHAW, A.B., Ph.D., Associate Professor of Anatomy, *University of Southern California, School of Medicine, Los Angeles 7, Calif.*

BARNARD, JOHN W., M.S., Ph.D., Associate Professor, *Department of Physiology, University of Wisconsin Medical School, Madison 6, Wisconsin.*

BARR, MURRAY L., B.A., M.D., M.Sc., Professor of Anatomy, Faculty of Medicine, *University of Western Ontario, 346 South Street, London, Canada.*

BARRETT, W. C., JR., Ph.D., Associate Professor of Anatomy, *School of Medicine, Boston University, 80 E. Concord St., Boston 18, Mass.*

BARRON, DONALD H., B.A., M.S., Ph.D., Associate Professor of Physiology, *Yale University School of Medicine, New Haven, Conn.*

BARRY, ALEXANDER, A.B., M.A., Ph.D., Associate Professor of Anatomy, *University of Michigan Medical School, Ann Arbor, Mich.*

BARTELMEZ, GEORGE W., Ph.D. (Vice-Pres. '32–'34, Exec. Com. '36–'40, President '48–'50), Professor Emeritus of Anatomy, The University of Chicago, Consultant, Dept. of Embryology, *Carnegie Institution of Washington, Wolfe and Madison Sts., Baltimore 5, Md.*

BARTSCH, PAUL, B.S., M.S., Ph.D., D.Sc., Curator of Mollusks and Cenozoic Invertebrates, *U. S. National Museum, Washington, D. C.*

BASSETT, DAVID L., A.B., M.D., Associate Professor of Anatomy, *Stanford University School of Medicine, Stanford, Calif.*

BAST, T. H., B.A., Ph.D., Professor of Anatomy, *University of Wisconsin, 308 Science Hall, Madison, Wis.*

BATES, M. NOBLE, A.B., A.M., Ph.D., Assistant Professor, Department of Anatomy, *School of Medicine, Temple University, Broad St. at Ontario, Philadelphia 40, Pa.*

BATSON, O. V., A.M., M.D., Professor of Anatomy, *Department of Anatomy, Graduate School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*

BAUMGARTNER, WILLIAM J., A.B., A.M., Ph.D., Professor of Zoology, University of Kansas, *1209 Ohio St., Lawrence, Kan.*

BEAMS, HAROLD W., Ph.D., Professor of Zoology, *State University of Iowa, Iowa City, Ia.*

Beaton, Lindsay E., A.B., M.S., M.D., Chief of Neuropsychiatry, Pima County Hospital; Consultant in Neuropsychiatry, Veterans Hospital; *1650 N. Campbell Ave., Tucson, Arizona.*

Beattie, John, M.D., D.Sc., Conservator of Museum and Director of Research, Royal College of Surgeons; Colonel, Army Medical Service; *Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2, England.*

Becker, R. Frederick, B.S., M.S., Ph.D., Associate Professor of Anatomy, *The Daniel Baugh Institute of Anatomy, 307 So. 11th St., Philadelphia, Pa.*

Bélanger, Leonard F., A.B., M.D., A.M., Professor of Histology and Embryology, *School of Medicine, University of Ottawa, Ottawa, Canada.*

Belou, Pedro, M.D., Titular Professor of Descriptive Anatomy Emeritus, Ex-Professor Titular de la Facultad de Ciencias *Medicas de Bs. Aires, Suipacha 856, Buenos Aires, Argentina.*

Benjamin, James W., A.B., M.S., Ph.D., Professor of Functional Anatomy, Director Department of Physiology-Pharmacology, *New York Medical College, 1 E. 105th St., New York City 29, New York.*

Bennett, George Allen, A.B., M.D., Dean, Professor of Anatomy and Director of the *Daniel Baugh Institute of Anatomy, Jefferson Medical College, 1025 Walnut St., Philadelphia, Pa.*

Bennett, Henry Stanley, A.B., M.D., Professor of Anatomy and the Head, Department of Anatomy, *School of Medicine, University of Washington, Seattle 5, Wash.*

Benoit, Jacques, M.D., Professeur d'Embryologie, *Institut d'Embryologie, Faculté de Medecine, Strasbourg, France.*

Bensley, Robert Russell, A.B., M.B., Sc.D. (Second Vice-Pres. '06–'07, Ex. Com. '08–'12, President '18–'20), Professor Emeritus of Anatomy, *The University of Chicago, Chicago, Ill.*

Bensley, Sylvia H., A.B., M.D., (Mrs. R.D.), Associate Professor of Anatomy Faculty of Medicine, *University of Toronto, Toronto 5, Ontario.*

Benton, Joseph G., A.B., M.Sc., Ph.D., M.D., Res. Associate, Physical Medicine and Rehab. N.Y.U. Bellevue Medical Center: Res. Ass't. Medicine N.Y.U., Goldwater Memorial Hospital, *New York University, College of Medicine, 477 First Ave., New York City 16, N. Y.*

Bergmann, Louis Lawrence, M.D., Associate Professor of Anatomy, *New York University College of Medicine, 477 First Avenue, New York 16, N. Y.*

Berry, Charles M., A.B., M.S., Ph.D., Assistant Professor of Anatomy, *Cornell University Medical College, 1300 York Ave., New York City.*

Best, R. Russell, B.Sc., M.D., Assistant Professor of Anatomy, Associate Professor of Surgery, University of Nebraska, College of Medicine, *527 Medical Arts Building, Omaha, Neb.*

Bevelander, Gerrit, Ph.D., Professor of Histology, *New York University, College of Dentistry, 477 First Ave., New York 16, N. Y.*

Bigelow, Robert P., S.B., Ph.D., Professor Emeritus of Zoology and Parasitology, Massachusetts Institute of Technology, *72 Blake Rd., Brookline, Mass.*

Bishop, Katherine Scott, A.B., M.D., *1508 La Loma Ave., Berkeley, Calif.*

Blair, Vilray Papin, M.D., A.B., Professor of Clinical Surgery, Washington University School of Medicine, *400 Metropolitan Building, St. Louis, Mo.*

Blaisdell, Frank Ellsworth, Sr., M.D., Emeritus Professor of Surgery, Medical Department of Stanford University, *1900 Washington St., San Francisco, Calif.*

Blandau, Richard J., A.B., Ph.D., M.D., Associate Professor of Anatomy, *School of Medicine, University of Washington, Seattle 5, Wash.*

Blincoe, Homer, A.B., M.S., M.D., Associate Professor of Anatomy, New York Medical College, *1 East 105th St., New York 29, N. Y.*

Bloch, Edward H., B.S., M.D., Ph.D., Established Investigator of the American Heart Association, *Western Reserve University School of Medicine, Cleveland, Ohio.*

Block, Matthew Harold, B.S., M.S., Ph.D., M.D., Assistant Professor in Medicine, *Department of Medicine, University of Chicago, Chicago 37, Ill.*

Bloom, William, A.B., M.D., (Exec. Com. '46–'50), Professor of Anatomy, *Department of Anatomy, The University of Chicago, Chicago, Ill.*

Blount, Isabel H., A.B., A.M., Ph.D., Voluntary Research Worker, *Department of Anatomy, University of Texas, Medical School, Galveston, Tex.*

Blount, Raymond Frank, M.S., Ph.D., Professor of Anatomy, *Department of Anatomy, University of Texas, Medical Branch, Galveston, Tex.*

Blumenfeld, Charles M., Ph.D., M.D., Pathologist, Sutter Hospital, *1433 41st Street, Sacramento 16, California.*

Bodian, David, S.B., Ph.D., M.D., Associate Professor of Epidemiology, *Johns Hopkins University School of Hygiene and Public Health, Poliomyelitis Laboratory, 1901 E. Madison Street, Baltimore 5, Md.*

Bogart, Ralph, B.S., M.S., Ph.D., Professor of Animal Husbandry, *Animal Husbandry Department, Oregon State College, Corvallis, Oregon.*

Boling, John L., Ph.D., Professor of Biology, *Linfield College, McMinnville, Oregon.*

Boling, Leroy Robb, A.B., M.S., Ph.D., Professor of Anatomy, *Washington University School of Dentistry, 4559 Scott Ave., St. Louis 10, Mo.*

Bonin, Wilbrod, B.A., M.D., Professor of Histology and Embryology, *Faculty of Medicine, Université de Montréal, 2900 Boulevard du Mont-Royal, Montréal, Canada.*

Bowie, Donald J., B.Sc., Med., M.A., Ph.D., Associate Professor of Anatomy, *Medical College, Winnipeg, Manitoba, Canada.*

Bowles, Lester, L., A.B., M.D., Professor of Microscopic Anatomy, *Medical College of Georgia, Augusta, Ga.*

Boyd, Edith, M.D., Associate Professor of Physical Growth, *University of Colorado, School of Medicine, 4200 E. Ninth Ave., Denver 7, Colorado.*

Boyd, James Dixon, M.A., M.Sc., M.D., Professor of Anatomy, *London Hospital Medical College, Turner Street, London, E.1, England.*

Boyden, Edward Allen, A.M., Ph.D. (Ex. Com. '28–'32, '39–'46, 1st Vice-Pres. '46–'48), Professor and Head of Dept. of Anatomy, *Institute of Anatomy, University of Minnesota, Minneapolis, Minn.*

Brachetto-Brian, Domingo, M.D., Director del Instituto de Medicina Experimental para el Estudio y Tratamiento del Cáncer, *Charcas 2508, Buenos Aires, Argentina.*

Bradbury, James T., B.S., M.S., Sc.D., Associate Professor of Obstetrics and Gynecology, University of Louisville, School of Medicine, *101 W. Chestnut St., Louisville 2, Ky.*

Bradley, E. Morton, B.A., Ph.D., Associate Professor of Histology, Southwestern Medical College, *2211 Oak Lawn Ave., Dallas 4, Texas.*

Brashear, Alton D., A.B., B.S., M.S., D.D.S., F.A.C.D., Associate Professor of Anatomy, *Department of Anatomy, Medical College of Virginia, Richmond 19, Va.*

Bremer, John Lewis, A.B., M.D. (Ex. Com. '15–'18, '33–'37), Hersey Professor of Anatomy Emeritus, Harvard University; *Children's Hospital, 300 Longwood Ave., Boston, Mass.*

Brewer, John I., S.B., M.D., Ph.D., Associate in Gynecology, Northwestern University Medical School and St. Luke's Hospital, Chicago, *104 S. Michigan, Chicago, Ill.*

Brizzee, Kenneth R., B.S., M.S., Ph.D., Instructor in Anatomy, *University of Utah College of Medicine, Salt Lake City, Utah.*

Brodie, Allan G., D.D.S., Ph.D., Professor of Orthodontia and Head of the Department, *College of Dentistry, University of Illinois, 30 N. Michigan Ave., Chicago, Ill.*

Browman, Ludvig G., Ph.D., Professor of Zoology and Physiology Chairman, Department of Zoology, *Montana State University, Missoula, Mont.*

Brown, James O., B.S., M.S., Ph.D., Assistant Professor of Anatomy, *The Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia, Pa.*

Browning, Henry Charles, B.Sc., Ed.Dip., Ph.D., Assistant Professor of Anatomy and Research Associate in Pathology, *Yale University School of Medicine, 310 Cedar St., New Haven, Conn.*

Brues, Alice M., A.B., Ph.D., Assistant Professor of Anatomy, *University of Oklahoma School of Medicine, 801 N.E. 13th Street, Oklahoma City, Okla.*

Brues, Austin Moore, A.B., M.D., Director, Division of Biological and Medical Research, Argonne National Laboratory, Chicago, and Associate Professor of Medicine, University of Chicago, *P.O. Box 5207, Chicago 80, Illinois.*

Bruesch, Simon Rulin, A.B., M.S., M.B., Ph.D., M.D., Professor of Anatomy, *University of Tennessee College of Medicine, Memphis, Tenn.*

Brunst, Victor V., Dr. Biol. Sc., Research Associate, School of Medicine, University of Maryland, *5803-C Oakview Ave., Baltimore 14, Maryland.*

Buchanan, A. R., B.S., M.S., M.D., Professor of Anatomy and Assistant Dean, *University of Colorado Medical School, 4200 E. Ninth Ave., Denver, Colo.*

Bueker, Elmer D., M.A., Ph.D., Associate Professor of Anatomy, *School of Medicine, University of Missouri, Columbia, Missouri.*

Bullard, H. Hays, A.M., Ph.D., M.D., *6700 Offutt Lane, Chevy Chase 15, Md.*

Bullock, Theodore Holmes, A.B., Ph.D., Associate Professor of Zoology, *University of California, Los Angeles 24, Calif.*

Buño, Washington, M.D., Professor of Histology and Embryology, *Department of Histology, Facultad de Medicina, Montevideo, Uruguay.*

Bunting, Charles Henry, B.S., M.D., Professor of Pathology, *139 Armory Street, Hamden, Connecticut.*

Burack, Ethel, Ph.D., M.D., Associate Professor of Anatomy, *Albany Medical College, Union University, Albany, N. Y.*

BURNS, B. I., A.B., A.M., Ph.D., M.D., Commissioner of Hospitals, Kansas City, Missouri, *Kansas City General Hospital, 24th and Cherry Sts., Kansas City, Missouri.*

BURNS, ROBERT K., A.B., Ph.D., Staff Member, Carnegie Institution of Washington and Honorary Professor of Zoology, Johns Hopkins University, Department of Embryology, *Carnegie Institution of Washington, Wolfe and Madison Sts., Baltimore 5, Md.*

BURR, HAROLD SAXTON, Ph.D., (Ex. Com. '29–'33), E. K. Hunt Professor of Anatomy, Head of Section of Neuro-Anatomy, School of Medicine, Yale University, *333 Cedar St., New Haven, Conn.*

BUTCHER, EARL O., A.B., M.A., Ph.D., Professor of Anatomy, *College of Denistry, New York University, 477 First Ave., New York City.*

BUTLER, ELMER G., A.M., Ph.D., Sc.D., Professor of Zoology, *Department of Biology, Princeton University, Princeton, N. J.*

CALHOUN, MARY LOIS, B.S., M.S., D.V.M., Ph.D., Professor and Head of Anatomy Department, *Anatomy Building, Michigan State College, East Lansing, Mich.*

CALKINS, LEROY A., M.D., M.S., Ph.D., Professor of Obstetrics and Gynecology, *University of Kansas Hospital, Kansas City, Kan.*

CAMERON, ANGUS L., A.B., M.S., M.D., Ph.D., *Northwest Clinic, Minot, N. Dak.*

CAMERON, JOHN A., A.B., A.M., Ph.D., Professor of Anatomy and Chairman of the Department, *Baylor University, College of Dentistry, Dallas 1, Tex.*

CAMPBELL, BERRY, A.B., Ph.D., Associate Professor of Anatomy, *Medical School, University of Minnesota, Minneapolis, Minn.*

CANDELA, POMPEO B., M.D., Research Associate, Dept. of Anatomy, New York Medical College; *1094 D Street, San Bernardino, Calif.*

CARPENTER, ESTHER, B.A., M.S., Ph.D., Associate Professor of Zoology, *Burton Hall, Smith College, Northampton, Mass.*

CARPENTER, MALCOLM BRECKENRIDGE, B.A., M.D., Research Fellow in Neurology, *College of Physicians and Surgeons, 630 West 168th St., New York 32, New York.*

CARPENTER, RUSSELL L., B.S., Ph.D., Professor of Zoology, *Tufts College, Medford, Mass.*

CARVER, GAIL L., A.B., A.M., Professor of Biology, *Mercer University, Macon, Ga.*

CASAMAJOR, LOUIS, A.M., M.D., Professor of Clinical Neurology, *Columbia University, 706 West 168th St., New York City.*

CASEY, ALBERT E., M.D. Pathologist and Director of Laboratories, The Baptist Hospital, Birmingham, Ala.; Assoc. Prof. of Pathology, Medical College of Alabama; *2236 Highland Ave., Birmingham, Ala.*

CASIDA, L. E., B.S., A.M., Ph.D., Professor of Genetics, *Department of Genetics, University of Wisconsin, Madison 6, Wis.*

CASTELNUOVO, GINA, Ph.D., Assistant Professor of Anatomy, *Woman's Medical College of Pennsylvania, Philadelphia, Pa.*

CAULDWELL, EARL WILLIAM, B.S., M.S., M.D., Assistant Professor of Pathology, *University of Illinois College of Medicine, 1853 W. Polk St., College of Medicine, Chicago, Ill.*

CHAMBERS, EDWARD LUCAS, A.B., M.D., Assistant Professor of Anatomy, The Johns Hopkins University School of Medicine, Department of Anatomy, *710 N. Washington St., Baltimore 5, Maryland.*

Chambers, Robert, Jr., M.A., Ph.D., LL.D. (hon.), (Second Vice-Pres. '39–'40), Research Professor Emeritus, New York University; Chief, *Laboratory of Experimental Cell Research, Marine Biological Laboratory, Woods Hole, Mass.*

Chambers, William Wallace, Jr., B.S., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Pennsylvania, School of Medicine, Philadelphia 4, Pa.*

Chandler, Simon B., A.B., A.M., M.D., Professor of Anatomy, *West Virginia University School of Medicine, Morgantown, W. Va.*

Chang, Chun, M.D., Professor of Anatomy, *Peiping Union Medical College, c/o Thos. Cook and Son, Hong Kong, China.*

Chang, Tso Kan, Ph.D., Assistant Professor of Anatomy, *Peiping Union Medical College, c/o Thos. Cook and Son, Hong Kong, China.*

Charipper, Harry A., B.S., M.S., Ph.D., Professor of Biology (Tissue Cytology, Endocrinology, Histology and Comparative Haematology), Chairman of Department, *Washington Square College, New York University, New York City.*

Charles, Cecil M., A.B., M.A., Ph.D., M.D., Associate Professor of Anatomy, *Washington University School of Medicine, St. Louis, Mo.*

Chase, Philena Evans, B.A., Ph.D., *320 Olive St., St. Louis, Mo.*

Chase, Ralph E., A.B., B.Sc., M.A., Instructor in Anatomy, *Oklahoma University Medical School, Oklahoma City, Okla.*

Chase, Samuel Wood, A.B., A.M., Ph.D., Associate Professor of Histology and Embryology, *Western Reserve University, 2109 Adelbert Road, Cleveland 6, O.*

Cherner, Maxwell, M.D., Police and Fire Surgeon, Dept. of Public Safety, Phila., Medical Examiner, Reading Railroad, *34 W. Phil-Ellena Street, Philadelphia 19, Pa.*

Chevremont, Maurice Jean Joseph, M.D., Professor of Histology and Embryology, *Institut d'Anatomie, University of Liège, 20, Rue de Pitteurs, Liège, Belgium.*

Child, Charles Manning, M.S., Ph.D., D.Sc., Professor Emeritus of Zoology, *Jordan Hall, Stanford University, Calif.*

Chouké, Kehar S., M.A., M.D., Associate Professor of Anatomy, *Graduate School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*

Christensen, Kermit, Ph.D., Associate Professor of Microanatomy, *St. Louis University School of Medicine, 1402 South Grand Blvd., St. Louis, Mo.*

Clark, Eleanor Linton, A.M., Voluntary Research Worker, Department of Anatomy, Medical Department, University of Pennsylvania; *Wistar Institute of Anatomy and Biology, Woodland Ave. and 36th St., Philadelphia 4, Pa.*

Clark, Eliot R., A.B., M.D., Sc.D., (Ex. Com. '16–'19, Sec.-Treas. '38–'42; Sec.-Treas. '43–'46), Professor of Anatomy Emeritus, Medical Department, University of Pennsylvania; *Wistar Institute of Anatomy and Biology, Woodland Ave and 36th St., Philadelphia 4, Pa.*

Clark, George, Ph.D., *Department of Anatomy, Chicago Medical School, 710 S. Wolcott St., Chicago, Illinois.*

Clark, Sam L., M.S., Ph.D., M.D., (Ex. Com. '42–'49, Pres. '50–), Professor of Anatomy, *Vanderbilt University School of Medicine, Nashville, Tenn.*

Clausen, Harry J., A.B., M.S., Ph.D., Associate Professor of Anatomy, *University of South Dakota School of Medicine, Vermillion, S. Dak.*

CLEMENTS, LEO, Ph.D., Associate Professor of Anatomy, Member Nebraska Basic Science Board, *Creighton University Medical School, Omaha, Neb.*

CLEVELAND, RUCKER, Ph.D., (Mrs. Ralph P. Rice), Instructor, Institutum Divi Thomae; medical writer (free lance), *303 Greenup, Covington, Ky.*

COBB, W. MONTAGUE, A.B., M.D., Ph.D., Professor of Anatomy, *Howard University, 520 W. St., N. W., Washington, D. C.*

COE, WESLEY R., Ph.D., Professor of Biology Emeritus, *Yale University, Osborn Zoological Laboratory, New Haven, Conn.*

COHN, ALFRED E., A.B., M.D., Member, *Rockefeller Institute for Medical Research, New York City, N. Y.*

COIDAN, ROSETTE SPOERRI, B.A., M.Sc., Ph.D., Instructor, Department of Anatomy, *State University of New York, State University Medical Center at New York City, College of Medicine, 350 Henry Street, Brooklyn 2, New York.*

COLE, ARCH EVAN, B.A., Ph.D., Professor of Anatomy, *University of Louisville; School of Medicine, 101 W. Chestnut St., Louisville, Ky.*

COLE, ELBERT C., Ph.D., Professor of Biology, *Williams College, Williamstown, Mass.*

COLE, HAROLD H., Ph.D., Professor of Animal Husbandry, University of California, and Animal Husbandman, *Experimental Station of the College of Agriculture, Davis, Calif.*

COLLINGS, M. RAYMOND, A.B., M.D., Professor of Anatomy, Medical College, Wayne University, *1512 St. Antoine St., Detroit, Mich.*

COMAN, DANA, A.B., A.M., M.D., LL.D., Consultant, War Department General Staff; Chairman, Panel on Expeditions, Research and Development Board, Washington, D. C.; *145 West 57th St., New York, N. Y.*

COMBS, CLARENCE MURPHY, M.S., B.A., Ph.D., Instructor, *Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill.*

CONEL, JESSE LEROY, A.M., Ph.D., Professor of Anatomy, Emeritus, Research Associate in Pathology, Boston University School of Medicine, *26 Richardson St., Newton 58, Mass.*

CONGDON, EDGAR DAVIDSON, Ph.D., Professor of Anatomy, Department of Anatomy, Chicago Medical School, *211 N. Oak Park Ave., Oak Park, Illinois.*

CONKLIN, EDWIN GRANT, A.M., Ph.D., Sc.D., L.L.D., Professor Emeritus of Biology, Princeton University, *139 Broadmead Ave., Princeton, N. J.*

COOPER, ZOLA K., A.B., M.S., Ph.D., Assistant Professor, Department of Pathology, *Washington University, School of Medicine, St. Louis 10, Mo.*

COPENHAVER, WILFRED M., A.B., Ph.D., Associate Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 632 West 168th St., New York City.*

COPELAND, DONALD EUGENE, A.B., M.A., Ph.D., Assistant Professor of Zoology, *Arnold Biological Laboratory, Brown University, Providence 12, RhodeIsland.*

CORBIN, KENDALL B., A.B., M.D., Associate Director Mayo Foundation, Professor of Neuroanatomy, Mayo Foundation, University of Minnesota, *Mayo Clinic, Rochester, Minn.*

COREY, EDWARD LYMAN, A.B., Ph.D., Professor of Physiology, University of Virginia Medical School, *Charlottesville, Va.*

Corner, George W., A.B., M.D., D.Sc., (Ex. Com. '26–'30, '38–'42; Sec.-Treas. '30–'38; Pres. '46–'48), Director, Department of Embryology, *Carnegie Institution of Washington, Wolfe and Madison Sts., Baltimore 5, Md.*

Cornman, Ivor, A.B., M.S., Ph.D., Assistant Research Professor in Anatomy, *George Washington University School of Medicine, 1339 H Street, N.W., Washington 5, D. C.*

Count, Earl W., A.B., B.D., Ph.D., Professor of Anthropology, *Department of Anthropology, Hamilton College, Clinton, N. Y.*

Covell, W. P., M.S., Ph.D., M.D., Associate Professor of Otolaryngology, *Department of Anatomy, Washington University, School of Medicine, St. Louis 10, Mo.*

Cowdry, Edmund V., Ph.D. (Ex-Com. '26–'30, 1st Vice-Pres. '34–'36), Research Professor of Anatomy, Director Wernse Cancer Research Laboratory, Washington University, *660 S. Kingshighway, St. Louis 10, Mo.*

Crafts, Roger Conant, B.S., Ph.D., Professor of Anatomy and Chairman of the Department, *College of Medicine, University of Cincinnati, Eden and Bethesda Avenues, Cincinnati 19, Ohio.*

Craigie, E. Horne, B.A., Ph.D. (Ex. Com. '41–'48), Professor of Comparative Anatomy and Neurology, *Department of Zoology, University of Toronto, Toronto 5, Canada.*

Crosby, Elizabeth Caroline, M.S., Ph.D., Professor of Anatomy, University of Michigan, *Department of Anatomy, University of Michigan, Ann Arbor, Mich.*

Crouch, Richard L., Ph.D., M.D., Associate Professor of Anatomy, *University of Missouri, Columbia, Mo.*

Cuajunco, Fidel, M.D., Associate Professor of Anatomy, College of Medicine, University of the Philippines, *620 Colorado St., Manila, P. I.*

Cullen, Thomas S., M.B., D.Sc., LL.D. (Tor.), Emeritus Professor of Gynecology, Johns Hopkins University, Visiting Gynecologist to the Johns Hopkins Hospital, *20 E. Eager St., Baltimore, Md.*

Cummins, Harold, A.B., Ph.D. (Ex. Com. '36–'40), Professor of Anatomy and Chairman of the Department, *Tulane University, Department of Anatomy, New Orleans 18, La.*

Cunningham, Robert S., B.S., A.M., M.D., Sc.D. (Ex. Com. '32–'36), Professor of Histology and Dean, Albany Medical College; Administrator, Albany Hospital, *47 New Scotland Ave., Albany 3, N. Y.*

Curtis, George M., A.M., Ph.D., M.D., Professor of Surgery and Chairman of the Department of Research Surgery; *Kinsman Hall, Ohio State University, Columbus, O.*

Curwen, Alice O., Ph.D. (Mrs. Edward F. McKeen), *Winterport, Maine.*

Cutuly, Eugene, B.S., M.S., Ph.D., Pfc. U. S. Army, *129 Constitution Circle, Clairton, Pa.*

Dalton, Albert J., B.S., M.A., Ph.D., Principal Cytologist, *National Cancer Institute, National Institute of Health, Bethesda 14, Md.*

Danforth, Charles Haskell, A.B., A.M., Ph.D., Sc.D., (Ex. Com. '27–'31, Second Vice-Pres. '36–'38), Professor of Anatomy, *Stanford University, Stanford University, Calif.*

D'Angelo, Savino Albert, B.A., M.A., Ph.D., Assistant Professor of Anatomy, Anatomy Department, *Daniel Baugh Institute, 307 S. 11th St., Philadelphia, Pa.*

Daron, Garman H., B.S., Ph.D., Associate Professor of Anatomy, *University of Oklahoma, School of Medicine, Oklahoma City, Okla.*

Davenport, H. A., M.D., Professor of Anatomy, Assistant Dean, *Northwestern University Medical School, Chicago, Ill.*

Davis, Carl L., M.D., Professor of Anatomy, University of Maryland School of Medicine, *Lombard and Greene Sts., Baltimore, Md.*

Dawson, Alden B., A.B., Ph.D., D.Sc., Professor of Zoology, *Biological Laboratories, Harvard University, Cambridge 38, Mass.*

Dawson, Helen L., B.S., M.S., Ph.D., Assistant Professor of Anatomy, *The State University of Iowa, College of Medicine, Iowa City, Ia.*

Day, Cameron D., Ph.D., Professor of Zoology, *Westminster College, Fulton, Mo.*

Deane, Helen Wendler, B.A., M.A., Ph.D., Assistant Professor of Anatomy, *Harvard Medical School, Boston, Mass.*

De Bruyn, Peter P. H., M.D., Associate Professor and Chairman, Department of Anatomy, *University of Chicago, Chicago 37, Ill.*

DeCarlo, John, M.D., formerly Associate in Topographic and Applied Anatomy, Jefferson Medical College, *1629 S. Broad St., Philadelphia 48, Pa.*

Dees-Mattingly, Marie, A.B., B.S., M.D., Associate Professor of Anatomy, *Tulane University of Louisiana, New Orleans, La.*

De Garis, Charles F., M.D., Ph.D., F.A.C.P., Professor of Anatomy, *University of Oklahoma, School of Medicine, Oklahoma City, Okla.*

Deignan, Stella L., B.S., M.S., Ph.D., Consultant, Work Projects Administration, *2236 Decatur Place, N. W., Washington 8, D. C.*

DeMarsh, Quin B., B.S., M.S., B.M., M.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Washington, School of Medicine, Seattle 5, Wash.*

Dempsey, Edward W., A.B., Sc.M., Ph.D., A.M. (Hon.), Professor of Anatomy, *School of Medicine, Washington Univ., 4580 Scott Ave., St. Lous 10, Mo.*

Dempster, Wilfrid Taylor, ScD., Associate Professor of Anatomy, *University of Michigan Medical School, Ann Arbor, Mich.*

Dent, James N., A.B., Ph.D., Associate Professor Biology, *Miller School of Biology, University of Virginia, Charlottesville, Va.*

Derbyshire, Arthur J., Ph.D., Assistant Professor of Physiology, *Harper Hospital, Detroit 1, Michigan.*

de Robertis, Eduardo D. P., M.D., Research Associate, *Instituto de Investigacion de Ciencias Biologicas, Avda Italia 3318, Montevideo, Uruguay.*

Detwiler, Samuel Randall, Ph.B., A.M., Ph.D., M.S. (Hon.), (Ex. Com. '30–'34), Professor of Anatomy, Executive Officer of the Department, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York 32, N. Y.*

Dey, Frederick L., A.B., M.S., Ph.D., Research Fellow, Institute of Neurology, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*

deMello, Jose P. P., M.D., Associate Professor of Anatomy, Chief of Practical Histology, University of Brazil.

Doan, Charles A., M.D., B.S., Dean, College of Medicine, and Director of Medical Research, *Ohio State University, Columbus, O.*

Dodds, Gideon S., M.A., Ph.D., Professor of Histology and Embryology, *West Virginia University Medical School, Morgantown, W. Va.*

Domm, L. V., A.B., Ph.D., Associate Professor of Anatomy, *Hull Anatomical Laboratory, The University of Chicago, Chicago 37, Ill.*

Donaldson, John C., Ph.B., M.D., Professor of Anatomy, *School of Medicine, University of Pittsburgh, Pittsburgh, Pa.*

Dougherty, Thomas F., Ph.D., Professor of Anatomy, *Department of Anatomy, University of Utah, Salt Lake City, Utah.*

Dow, Robert S., A.B., M.A., M.D., Ph.D., Assistant Clinical Professor of Neurology, *University of Oregon Medical School, 3181 S.W. Morgan Hill Rd., Portland, Ore.*

Downey, Hal, A.M., Ph.D., Professor Emeritus of Anatomy, *University of Minnesota Medical School, Minneapolis, Minn.*

Doyle, William Lewis, M.A., Ph.D., Professor of Anatomy, *Hull Anatomical Laboratory, University of Chicago, Chicago 37, Ill.*

Drager, Glenn A., B.S., M.S., Ph.D., Associate Professor of Neuroanatomy, Dept. of Anatomy, *University of Texas, Medical Branch, Galveston, Tex.*

DuBois, Franklin S., A.B., M.S., M.D., Associate Medical Director, Silver Hill Foundation, *Silver Hill, New Canaan, Conn.*

DuBrul, E. Lloyd, D.D.S., M.S., Assistant Professor in Charge of Division of Oral Anatomy, *University of Ilinois, College of Medicine, Division of Oral Anatomy, 808 South Wood Street, Chicago, Illinois.*

Duke, Kenneth L., A.B., Ph.D., Associate Professor of Anatomy, *Duke University School of Medicine, Durham, N. C.*

Duncan, Donald, B.A., M.A., Ph.D., (Exec. Com. '50–), Professor of Anatomy, and Chairman of the Department, *School of Medicine, University of Texas, Galveston, Texas.*

Dunihue, Fred Williams, A.B., M.Sc., Ph.D., Professor of Histology and Embryology, *Medical College, University of Vermont, Burlington, Vt.*

Duryee, William Rankin, A.B., Ph.D., Staff Associate at the Carnegie Institution of Washington; Cytologist at the National Cancer Institute, *Department of Terrestrial Magnetism, 5241 Broad Branch Road, Washington, D. C.*

DuShane, Graham Phillips, A.B., Ph.D., Professor of Biology, *Department of Biology, Stanford University, Calif.*

Dvoskin, Samuel, Ph.D., M.D., Assistant in Medicine, College of Physicians and Surgeons, Columbia University, N. Y., *200 Carbini Blvd., New York 33, N. Y.*

Earle, Wilton R., Ph.D., Principal Cytologist, *National Cancer Institute, Bethesda, Md.*

Eastlick, Herbert L., A.B., M.S., Ph.D., Professor of Zoology and Chairman of the Department, *State College of Washington, Pullman, Wash.*

Edds, Mac Vincent, Jr., B.A., M.A., Ph.D., *Biology Department, Brown University, Providence 12, R. I.*

Edwards, Edward A., M.D., Clinical Associate in Anatomy, *Harvard Medical School, Boston, Mass.*

Edwards, J. Graham, M.A., Ph.D., Associate Professor of Anatomy, University of Buffalo School of Medicine, *24 High St., Buffalo, N. Y.*

Edwards, Jesse E., B.S., M.D., Assistant Professor, Pathologic Anatomy, Graduate School, University of Minnesota, Mayo Foundation and Consulting Physician, *Mayo Clinic, Rochester, Minn.*

Edwards, Linden F., B.A., M.S., Ph.D., Professor of Anatomy, Ohio State University College of Medicine, *Hamilton Hall, Columbus 10, O.*

Elftman, Alice G., B.A., M.A., Ph.D., Assistant Professor of Biological Sciences, *Hunter College, New York City; 695 Park Ave., New York 21, N. Y.*

Elftman, Herbert, Ph.D., Associate Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*

Elias, M. Hans, Ph.D., Assistant Professor of Anatomy, *The Chicago Medical School, 710 South Wolcott Ave., Chicago 12, Ill.*

Eliot, Theodore Sessinghaus, A.B., A.M., Ph.D., Associate Professor of Anatomy, *School of Medicine, University of Colorado, 4200 East 9th Ave., Denver 7, Colo.*

Elliott, E. Chandler, M.A., Ph.D., *University of Nebraska School of Medicine, Omaha, Neb.*

Elliott, Rush, A.B., A.M., Ph.D., Professor of Anatomy, Department of Zoology, Ohio University, *3 Marietta Ave., Athens, O.*

Elwyn, Adolph, A.M., Associate Professor of Neuroanatomy, *Columbia University, 630 West 168th St., New York City.*

Ely, Charles A., A.B., M.S., Ph.D., Associate in Anatomy, *College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York 32, New York.*

Emery, Frederick E., Ph.D., Professor of Physiology, *Department of Physiolgy, University of Arkansas School of Medicine, Little Rock, Ark.*

Emmel, Victor M., Ph.D., M.D., Associate Professor of Anatomy, *The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*

Enders, Robert K., A.B., Ph.D., Professor and Chairman, Zoology Department, *Swarthmore College, Swarthmore, Pa.*

Engle, Earl Theron, A.B., A.M., Ph.D., Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 632 West 168th St., New York City.*

Essenberg, J. M., B.S., Ph.B., Ph.D., Professor of Anatomy, *The Chicago Medical College, 710 S. Wolcott Ave., Chicago, Ill.*

Essick, Charles Rhein, B.A., M.D., *700 Centre Ave., Reading, Pa.*

Etkin, William, Ph.D., Associate Professor of Biology, *College of the City of New York, New York City 31, N. Y.*

Ettinger, George Harold, M.B.E., B.A., M.D.C.M., F.R.S.C., Assistant Director, Division of Medical Research, National Research Council, Ottawa; Professor of Physiology, *Queen's University, Kingston, Ontario, Canada.*

Evans, F. Gaynor, B.A., M.A., Ph.D., Associate Professor of Anatomy, *Wayne University, College of Medicine, Detroit 26, Mich.*

Evans, Herbert McLean, B. S., M.D., Sc.D., Docteur, honoris causa (Paris), (Ex. Com. '19–'22; Pres. '30–'32), Professor of Anatomy, Professor of Biology and Director of the Institute of Experimental Biology, University of California, *Room 4579 Life Sciences Building, University of California, Berkeley 4, California.*

Evans, Llewellyn Thomas, A.B., A.M., Ph.D., Professor, Research Associate, American Museum of Natural History, *64 Walworth Ave., Scarsdale, New York.*

EVANS, THOMAS HORACE, M.D., Research Professor of Anatomy and Lecturer to School of Nursing, New York Medical College and Flower-Fifth Ave. Hospital, New York 29, N.W.; *350 South Main St., Freeport, N. Y.*

EVANS, VIRGINIA JOHN, B.A., M.Sc., Sc.D., Cytologist, *National Cancer Institute, Bethesda, Md.*

EVERETT, JOHN W., A.B., Ph.D., Professor of Anatomy, *Duke University School of Medicine, Durham, N. C.*

EVERETT, NEWTON B., B.S., M.S., Ph.D., Associate Professor of Anatomy, *University of Washington, School of Medicine, Seattle 5, Washington.*

EVERSOLE, WILBURN J., A.B., M.Sc., Ph.D., Assistant Professor, *Department of Zoology, Syracuse University, Syracuse, N. Y.*

FANKHAUSER, GERHARD, Ph.D., Professor of Biology, *Princeton University, Princeton, N. J.*

FARR, RICHARD STUDLEY, S.B., M.D., Lieutenant, U. S. Navy (Medical Corps), *National Naval Medical Center, Naval Medical Research Inst., Bethesda 14, Maryland.*

FARRIS, EDMOND J., Ph.D., Associate Member in Anatomy, Executive Director, *The Wistar Institute of Anatomy and Biology, Woodland Ave. and 36th St., Philadelphia 4, Pa.*

FAWCETT, DON WAYNE, A.B., M.D., Associate in Anatomy, Department of Anatomy, *Harvard University Medical School, 25 Shattuck Street, Boston, Massachusetts.*

FEKETE, ELIZABETH, B.A., M.A., Research Associate, *R. B. Jackson Memorial Laboratory, Bar Harbor, Me.*

FIGGE, F. H. J., Ph.D., Professor of Anatomy, *University of Maryland Medical School, 29 S. Green St., Baltimore 1, Maryland.*

FINERTY, JOHN C., A.B., M.S., Ph.D., Associate Professor of Anatomy, *University of Texas Medical Branch, Galveston, Texas.*

FITZGERALD, LAURENCE ROCKWELL, B.S., M.S., Ph.D., Instructor in Anatomy, *University of Tennessee, Memphis, Tenn.*

FLEXNER, LOUIS BARKHOUSE, S.B., M.D., Chairman Department of Anatomy and Professor of Anatomy, *School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*

FOGG, LLOYD C., B.S., M.S., Ph.D., Chief of Laboratory, Pondville State Hospital; Assistant in Pathology, Harvard Medical School, *17 Glenwood Ave., Newton Center 59, Mass.*

FOLEY, JAMES OWEN, M.S., Ph.D., Professor of Anatomy and Chairman, *Department of Anatomy, Medical College of Alabama, Birmingham 5, Ala.*

FORBES, THOMAS R., A.B., Ph.D., Assistant Dean, Assistant Professor of Anatomy, *Yale University, School of Medicine, 333 Cedar St., New Haven, Conn.*

FORMAN, JONATHAN, A.B., M.D., Professor Hist. Med., Lecturer on Diseases Due to Allergy, Ohio State University, *394 East Town St., Columbus, O.*

FOUST, H. L., D.V.M., Professor of Veterinary Anatomy, *Iowa State College, Ames, Ia.*

FOX, CLEMENT A., B.S., M.A., Ph.D., Professor of Anatomy, *Marquette University School of Medicine, Milwaukee 3, Wis.*

FRANCIS, C. C., M.D., Assistant Professor of Anatomy, *Western Reserve University, School of Medicine, Cleveland 6, O.*

Frazer, John Ernest, D.Sc., F.R.C.S., Professor of Anatomy, University of London, *St. Mary's Hospital Medical School, London, W., England.*

French, H. E., M.S., M.D., Professor of Anatomy and Dean of the School of Medicine, *University of North Dakota, Grand Forks, N. Dak.*

Friedman, Sydney M., M.D., Ph.D., Professor of Anatomy, *Department of Anatomy, The University of British Columbia, Vancouver, B. C.*

Furuta, William J., A.B., M.S., Ph.D., Assistant Professor of Anatomy, *College of Medicine, University of Illinois, 1853 West Polk Street, Chicago 12, Illinois.*

Gabriel, Mordecai L., B.A., M.A., Ph.D., Assistant Professor of Biology, *Brooklyn College, Bedford Ave. and Ave. H, Brooklyn 10, N.Y.*

Gamble, Dean L., B.S., Ph.D., President, Ward's Natural Science Establishment, *P. O. Box 24, Beechwood Station, Rochester, N. Y.*

Garcia, Arturo, A.B., M.D., Professor of Anatomy and Head of Department, College of Medicine, University of the Philippines (retired), *619 Colorado St., Manila, P. I.*

Gardner, Ernest, M.D., Professor of Anatomy and Chairman of the Department, *School of Medicine, Wayne University, Detroit 26, Michigan.*

Gardner, Weston D., B.S., M.D., Instructor in Anatomy, *Marquette University School of Medicine, 561 North 15th St., Milwaukee 3, Wisconsin.*

Gardner, William U., Ph.D., (Exec. Com. '48–), Professor of Anatomy and Chairman of the Department, *Yale University School of Medicine, 333 Cedar St., New Haven, Conn.*

Garrett, Frederic D., A.B., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, Hamilton Hall, Ohio State University, Columbus, Ohio.*

Gatz, Arthur J., B.A., M.A., Ph.D., Associate Professor of Anatomy, *Loyola University, Stritch School of Medicine, 706 So. Wolcott Ave., Chicago 12, Ill.*

Gaunt, Robert, Ph.D., Professor of Zoology and Chairman of the Department, *Lyman Hall, Syracuse University, Syracuse, N. Y.*

Geist, Frederick D., M.D., Associate Professor of Anatomy, *University of Wisconsin, Science Hall, Madison, Wis.*

Geohegan, William A., E.E., M.D., Assistant Professor, *Department of Anatomy, Cornell University Medical College, New York City 21, N. Y.*

George, Wesley Critz, A.B., A.M., Ph.D., Professor of Histology and Embryology, and Head of the Department of Anatomy, *University of North Carolina Medical School, Chapel Hill, N. C.*

Gersh, Isidore, A.B., Ph.D., Associate Professor, *Department of Anatomy, University of Chicago, Chicago 37, Ill.*

Getty, Robert, D.V.M., M.S., Ph.D., Associate Professor, *Iowa State College, Ames, Iowa.*

Gey, George O., B.S., M.D., Assistant Professor of Surgery, Johns Hopkins Hospital and University, *Johns Hopkins Medical School, Baltimore 5, Md.*

Gilbert, Perry W., A.B., Ph.D., Associate Professor of Zoology, *Department of Zoology, Cornell University, Ithaca, N. Y.*

Gillilan, Lois A., B.A., A.M., Ph.D., M.D., Assistant Professor of Anatomy, *Graduate School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*

Glickman, Irving, B.S., D.M.D., Assistant Professor of Oral Pathology, Tufts College Dental School, *416 Huntington Avenue, Boston, Mass.*

GLOBUS, J. H., B.S., M.D., Associate Professor of Neuro-Anatomy, New York University, *960 Park Ave., New York City.*

GLUECKSOHN-WAELSCH, SALOME, Ph.D., Research Associate, *Department of Zoology, Columbia University, New York 27, N. Y.*

GODDARD, JAMES WILLIAM, M.D., Research Associate in Pathology, Harvard Medical School, *Yamins Research Laboratory, Beth Israel Hospital, Boston, Mass.*

GODWIN, MELVIN, C., Ph.D., M.D., Resident Pathologist, *Veterans Administration Hospital, Hines, Illinois.*

GOLDSMITH, ELI D., M.A., M.S., Ph.D., Associate Professor of Histology, Research Coordinator, *New York University, College of Dentistry, 477 First Avenue, New York 16, N. Y.*

GOLDSMITH, JOSEPH B., M.A., Ph.D., Associate Professor of Histology and Embryology, *Oklahoma University School of Medicine, Oklahoma City, Okla.*

GOOD, ROBERT A., M.D., Ph.D., Helen Hay Whitney Fellow in Rheumatic Fever, *Dept. of Pediatrics, University of Minnesota, Minneapolis 14, Minn.*

GOODRICH, HUBERT BAKER, B.S., M.A., Ph.D., Professor of Biology, *Wesleyan University, Middletown, Conn.*

GORBMAN, AUBREY, A.B., M.S., Ph.D., Associate Professor of Zoology, *Barnard College, Columbia University, New York 27, N. Y.*

GORDON, ALBERT S., B.S., M.S., Ph.D., Associate Professor of Biology, *Washington Square College of Arts and Science, New York University, New York 3, N. Y.*

GOSS, CHARLES MAYO, A.B., M.D., (Exec. Com. '47–), Professor of Anatomy, *Louisiana State University School of Medicine, New Orleans 13, La.*

GOULD, HARLEY NATHAN, A.B., A.M., Ph.D., Professor of Biology, *Newcomb College, Tulane University, New Orleans, La.*

GRAFFLIN, ALLAN L., A.B., M.D., Professor of Anatomy and Director, Department of Anatomy, *The Johns Hopkins University School of Medicine, 710 N. Washington St., Baltimore 5, Maryland.*

GRANGER, BARBARA SAMSON, A.B., A.M., Ph.D., Assistant Professor of Anatomy, *University of Southern California, Los Angeles 7, Calif.*

GRANT, J. C. BOILEAU, M.B., Ch.B., F.R.C.S. (Edin.), (Sec. Vice Pres. '50–), Professor of Anatomy, *University of Toronto, Toronto, Canada.*

GRANT, MADELEINE P., S.B., M.A., Ph.D., Member Teaching Staff in Zoology, *Sarah Lawrence College, Bronxville, N. Y.*

GRAY, DONALD J., B.S., M.S., Ph.D., Professor of Anatomy, *Department of Anatomy, Stanford University, Stanford, California.*

GRAY, MARY E., B.A., Ph.D., Assistant Professor of Anatomy, *Vanderbilt University School of Medicine, Nashville, Tenn.*

GRAY, PETER, Ph.D., D.I.C., Professor and Head Dept. of Biological Sciences, *University of Pittsburgh, Pittsburgh 13, Pa.*

GRAY, STEPHEN WOOD, B.A., M.A., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, Emory University, Georgia.*

GREEN, EARL LEROY, B.S., ScM., Ph.D., Associate Professor of Zoology, Dept. of Zoology, *Ohio State University, Columbus 10, Ohio.*

GREEN, JOHN DAVIS, B.M., B.Ch., M.A., Instructor in Anatomy, *Wayne University College of Medicine, 1512 St. Antoine St., Detroit 26, Mich.*

GREENE, CHARLES W., A.M., Ph.D., Professor Emeritus of Physiology and Pharmacology, University of Missouri, *814 Virginia Ave., Columbia, Mo.*

GREENE, EUNICE CHASE, B.A., M.A., (Mrs. Walter F.) *Department of Biology, Springfield College, Springfield 9, Mass.*

GREENE, WALTER FARRER, A.B., Ph.D., *Department of Biology, Springfield College, Springfield 9, Mass.*

GREENHILL, J. P., B.S., M.D., F.A.C.S., Professor and Vice Chairman, Department of Obstetrics and Gynecology, Loyola University Medical School; Professor of Gynecology, Cook County Graduate School of Medicine; Attending Gynecologist, Cook County Hospital, *Suite 2805–06 Pittsfield Building, 55 East Washington St., Chicago, Ill.*

GREEP, ROY O., M.S., M.A. (hon.), Ph.D., Associate Professor of Dental Science, *Harvard School of Dental Medicine, 25 Shattuck St., Boston 15, Mass.*

GREGORY, WILLIAM KING, A.B., A.M., Ph.D., Sc.D., Da Costa Professor of Vertebrate Palaeontology, Columbia University; Curator Emeritus, Departments of Comparative Anatomy and Fishes, *American Museum of Natural History, New York City 24.*

GREULICH, WILLIAM W., Ph.B., M.A., Ph.D., (Exec. Com. '46–'50), Professor of Anatomy, Stanford University School of Medicine, *P.O. Box 1558, Stanford, California.*

GROAT, RICHARD A., B.A., M.S., Ph.D., Associate Professor of Anatomy, *Bowman Gray School of Medicine of Wake Forest College, Winston-Salem 7, North Carolina.*

GRODINSKY, MANUEL, B.Sc., M.D., Associate Professor of Anatomy and Surgery, University of Nebraska, College of Medicine, *Apt. 7, Casa Linda, 108 S. 49th Ave., Omaha, Neb.*

GROSS, JACK, B.Sc., M.D., Ph.D., Merck Fellow in Natural Sciences, *National Institute for Medical Research, The Ridgeway, Mill Hill, N.W. 7, London, England.*

GRUENWALD, PETER, M.D., Pathologist and Director of Laboratories, Beth-el Hospital; Clinical Assistant Professor of Obstetrics and Gynecology, State Hosp. of New York, *Beth-el Hospital, Brooklyn 12, New York.*

GUDERNATSCH, FREDRICK, Ph.D., Sc.D., (retired), *41 Fifth Ave., New York 3, N.Y.*

GUILD, STACY R., A.M., Ph.D. (Ex. Com. '39–'46), Associate Professor of Otology and Director of Otological Research Laboratory, Johns Hopkins University, *Johns Hopkins Hospital, Baltimore, Md.*

GURDJIAN, ELISHA STEPHENS, A.B., M.S., M.D., Ph.D., F.A.C.S., Neuro-Surgeon, Grace Providence and Receiving Hospitals; Instructor in Surgery, Wayne University, *840 David Whitney Bldg., Detroit, Mich.*

GUTHRIE, MARY J., A.B., A.M., Ph.D., Professor of Zoology, *210 Lefevre Hall, University of Missouri, Columbia, Mo.*

GUYER, MICHAEL F., Ph.D., LL.D., Professor of Zoology, *University of Wisconsin, Madison, Wis.*

HAHN, WILLIAM E., D.D.S., A.B., M.S., Professor of Anatomy, *School of Dentistry, University of Maryland, 618 W. Lombard St., Baltimore 1. Md.*

HALEY, JOHN CHAMBLESS, M.D., B.S., Professor of Anatomy and Chairman of Department, *Baylor University College of Medicine, Houston, Texas.*

HALL, B. VINCENT, A.B., Ph.D., Associate Professor of Zoology, *University of Illinois, Urbana. Ill.*

HALL, CHARLES ERIC, B.S., M.S., Ph.D., Associate Professor of Physiology, Consulting Endocrinologist, Medical Branch Hospital, *Department of Physiology, University of Texas, Medical Branch, Galveston, Texas.*

HALL, EDMUND K., D.Sc., Ph.D., Associate Professor of Anatomy, *University of Louisville Medical School, Louisville, Ky.*

HALL, E. RAYMOND, A.B., M.A., Ph.D., Professor of Zoology, Chairman Department of Zoology, Director Museum of Natural History, *University of Kansas, Lawrence, Kansas.*

HALPERT, BÉLA, M.D., Chief, Laboratory Service, Veterans Adm. Hospital and Assoc. Prof. of Pathology, Baylor University, College of Medicine, *Veterans Administration Hospital, 2002 Holcombe Blvd., Houston 4, Texas.*

HAM, ARTHUR W., M.B., Professor of Anatomy, in charge of Histology, *Department of Anatomy, University of Toronto, Toronto, Canada.*

HAMBLEN, E. C., M.D., Associate Professor of Obstetrics and Gynecology, *Duke University School of Medicine, Durham, N. C.*

HAMBURGER, VIKTOR, Ph.D., Professor of Zoology, *Washington University, St. Louis, Mo.*

HAMILTON, JAMES B., Ph.D., Professor of Anatomy, Long Island College of Medicine, *350 Henry St., Brooklyn 2, New York.*

HAMILTON, WILLIAM J., M.D., B.Ch., D.Sc., F.R.S.E., Professor of Anatomy, *University of London, Charing Cross Hospital Medical College, 62 Chandos Place, London, W.C.2., England.*

HAMLETT, GEORGE W. D., A.B., A.M., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, Louisiana State University School of Medicine, New Orleans 13, Louisiana.*

HAMMOND, WARNER S., Ph.D., Assistant Professor of Anatomy, *School of Medicine, State University of New York, Syracuse 10, N. Y.*

HAMRE, CHRISTOPHER J., B.A., M.S., Ph.D., Professor of Anatomy, *University of North Dakota, Grand Forks, North Dakota.*

HANAN, ERNEST B., A.B., A.M., M.D., Chief Pathologist of Burge Hospital School of Nursing, *600 Medical Arts Building, Springfield, Mo.*

HARD, WALTER LEON, A.M., Ph.D., Professor of Anatomy and Chairman, *Department of Anatomy, School of Medicine, University of S. Dakota, Vermillion, S. Dakota.*

HARDINGE, MERVYN GILBERT, B.S., M.D., M.P.H., Assistant Professor of Anatomy, *College of Medical Evangelists, Loma Linda, California.*

HARE, W. KENDRICK, A.B., M.S., Ph.D., M.D., Research Associate in Pediatrics, *Children's Hospital, 219 Bryant St., Buffalo 9, N. Y.*

HARGITT, GEORGE T., Ph.B., A.M., Ph.D., Sc.D., Professor of Zoology, *Biology Bldg., Duke University, Durham, N. C.*

HARMAN, PINCKNEY JONES, Ph.D., Associate Professor of Anatomy, *New York University College of Medicine, 477 First Ave., New York 16, N. Y.*

HARPER, WALTER FEARN, M.D., Ph.D., B.Sc., Professor of Anatomy, *University College of the West Indies, Mona, Jamaica.*

HARRIS, HENRY A., M.A., M.D., B.S., M.R.C.P., D.Sc., Professor of Anatomy, *The Anatomy School, The University, Cambridge, England.*

HARRIS, MORGAN, A.B., Ph.D., Associate Professor of Zoology, *University of California, Berkeley, Calif.*

Harrison, Bruce M., B.S., M.S., Ph.D., Sc.D., Professor of Zoology, Emeritus, *University of Southern California, Los Angeles 7, California.*

Harrison, Frank, B.S., M.S., Ph.D., Professor of Anatomy and Chief, *Division of Anatomy, University of Tennessee, Memphis 3, Tenn.*

Harrison, Ross Granville, Ph.D., M.D., Sc.D., LL.D., (Pres. '12–'14), Sterling Professor of Biology and Director of Osborn Zoological Laboratory Emeritus, *Yale University, New Haven, Conn.*

Hartman, Carl G., M.A., Ph.D. (Ex. Com. '29–'33) (First Vice-Pres. '40–'42), *Ortho Research Foundation, Raritan, New Jersey.*

Hartmann, J. Francis, A.B., Ph.D., Assistant Professor of Anatomy, *Institute of Anatomy, University of Minnesota, Minneapolis 14, Minn.*

Hartroft, Walter Stanley, B.Sc., M.D., Assistant Professor, *Banting and Best Department of Medical Research, Banting Institute, University of Toronto, 100 College Street, Toronto 2, Ontario, Canada.*

Harvey, Basil Coleman Hyatt, A.B., M.D., Professor of Anatomy Emeritus, *The University of Chicago, Chicago, Ill.*

Hashimoto, Edward I., A.B., M.D., M.S., Associate Professor of Anatomy, *University of Utah Medical School, Salt Lake City, Utah.*

Hausberger, Franz X., M.D., Assistant Professor of Anatomy, *The Jefferson Medical College, The Daniel Baugh Institute of Anatomy, 307 South Eleventh Street, Philadelphia 7, Pa.*

Hausman, Louis, A.B., M.D., Professor of Clinical Neurology, Cornell University Medical College; Associate Attending Physician, New York Hospital, *140 E. 54th St., New York 22, N. Y.*

Haymaker, Webb, M.D., M.Sc., Professorial Lecturer in Anatomy, George Washington University, School of Medicine; Chief, Neuropathology Section, *Armed Forces Institute, Washington 25, D. C.*

Healey, John Edward, Jr., B.S., M.D., Associate in Anatomy, *Daniel Baugh Institute of Anatomy, Jefferson Medical College, 307 So. 11th St., Philadelphia, Pa.*

Hegre, Erling S., B.A., M.S., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, Medical College of Virginia, Richmond 19, Virginia.*

Heinbecker, Peter, M.D., Professor of Clinical Surgery, *Washington University School of Medicine, Euclid and Kingshighway, St. Louis 10 Mo.*

Heldt, Thomas J., A.B., A.M., M.D., Physician in Charge of Division of Neuropsychiatry, *Henry Ford Hospital, Detroit, Mich.*

Helff, O. M., B.S., M.S., Ph.D., Associate Professor of Biology, *New York University, University Heights, New York City.*

Hellbaum, Arthur A., Ph.D., M.D., Professor of Pharmacology, *University of Oklahoma, School of Medicine, Oklahoma City, Okla.*

Herrick, Charles Judson, Ph.D., (Ex. Com. '13–'17), Emeritus Professor of Neurology, The University of Chicago, *236 Morningside Drive, Grand Rapids 6, Mich.*

Hertig, Arthur Tremain, B.S., M.D., Professor of Pathology, Harvard Medical School; Pathologist, Boston Lying-in Hospital; Pathologist, Free Hospital for Women, Brookline, Mass.; *221 Longwood Ave., Boston 15, Mass.*

Hetherington, Albert W., B.S., Ph.D., Chief, Dept. of Physiology, *U. S. A. F. School of Aviation Medicine, Randolph Field, Texas.*

HETHERINGTON, DUNCAN CHARTERIS, A.B., M.A., Ph.D., M.D., Professor of Anatomy, *Duke University Medical School, Durham, N. C.*

HEUSER, CHESTER H., A.B., A.M., Ph.D., Professor of Microscopic Anatomy, *Medical College of Georgia, Augusta, Georgia.*

HIGGINS, GEORGE M., A.M., Ph.D., Associate Professor of Experimental Biology, *University of Minnesota, Mayo Foundation, Rochester, Minn.*

HILL, JAMES PETER, D.Sc., F.R.S., Professor Emeritus of Embryology, Department of Anatomy, University College, London, W. C. 1, *Kanimbla Dollis Ave., Finchley, London, W. 3, England.*

HILL, ROBERT T., Ph.D., Professor of Anatomy, Medical Research Unit, *The University of Miami, Veterans' Administration Hospital, Coral Gables 34, Florida.*

HILTON, WILLIAM A., B.S., Ph.D., Professor of Zoology, Pomona College, Director Laguna Marine Laboratory, *1263 Dartmouth Ave., Claremont, Calif.*

HINES, MARION, A.B., Ph.D., Sc.D., (2nd Vice-Pres. '46–'48), Professor of Experimental Anatomy and Research Assistant in Physical Medicine, *Emory University, Georgia.*

HINSEY, JOSEPH C., M.S., Ph.D., (Ex. Com. '40–'46), Dean and Professor of Anatomy, *Cornell University Medical College, 1300 York Ave., New York City 21.*

HIRSCH, G. CHRISTIAN, Ph.D., *Hainholzweg 66, Göttingen (206), Germany.*

HISAW, FREDERICK L., A.B., A.M., Ph.D., LL.D., Professor of Zoology, Chairman of the Department of Biology, *Biological Laboratories, Harvard University, Cambridge, Mass.*

HOADLEY, LEIGH, A.B., Ph.D., Professor of Zoology, *Harvard University, Cambridge, Mass.*

HOERR, NORMAND L., A.B., Ph.D., M.D., (Sec.-Treas. '46–) Henry Willson Payne, Professor of Anatomy, *School of Medicine, Western Reserve University, 2109 Adelbert Rd., Cleveland 6, O.*

HOGG, IRA D., A.B., M.A., Ph.D., Professor of Anatomy, Head of Department of Anatomy, *University of Mississippi, Box 102, University, Miss.*

HOGUE, MARY J., Ph.D., Associate Professor of Anatomy, *University of Pennsylvania Medical School, Philadelphia 4, Pa.*

HOLLINSHEAD, W. HENRY, A.B., M.S., Ph.D., Professor of Anatomy, Mayo Foundation and Head of the Section on Anatomy, *Mayo Clinic, Rochester, Minn.*

HOLT, CAROLINE M., A.B., A.M., Ph.D., *8 Norfolk Terrace, Wellesley, Mass.*

HOLYOKE, EDWARD A., M.D., Ph.D., Professor of Anatomy, *University of Nebraska, College of Medicine, 42nd and Dewey Ave., Omaha, Neb.*

HOOKER, CHARLES W., A.B., A.M., Ph.D., Professor of Anatomy, *The University of North Carolina, Chapel Hill, N. C.*

HOOKER, DAVENPORT, Ph.D., Sc.D. (Ex. Com., '22–'25) (Second Vice-Pres. '40–'42), Professor of Anatomy and Head of the Department, *School of Medicine, University of Pittsburgh, Pittsburgh 13, Pa.*

HOPKINS, GRANT SHERMAN, Sc.D., D.V.M., Emeritus Professor of Comparative Veterinary Anatomy, *Cornell University, Ithaca, N. Y.*

HOUSE, EARL LAWRENCE, B.S., M.A., Ph.D., Assistant Professor of Anatomy, *New York Medicine College, Flower and Fifth Ave. Hospitals, New York 29, N. Y.*

HOWE, HOWARD A., M.D., Adjunct Professor of Epidemiology, *Johns Hopkins University School of Hygiene, Baltimore 5, Md.*

HOWLAND, RUTH B., Ph.D., Ph.M., Professor of Biology, Washington Square College, *New York University, Washington Square East, New York City.*

HOY, WM. E., A.B., Ph.D., Professor of Biology and Head of the Department of Biology, *University of South Carolina, Columbia, S. C.*

HUBER, JOHN FRANKLIN, A.M., M.D., Ph.D., Professor and Head of the Department of Anatomy, *School of Medicine, Temple University, Broad St. at Ontario, Philadelphia 40, Pa.*

HUMM, FRANCES DORRIS, A.B., A.M., Ph.D., Research Assistant in Pharmacology, *Yale University, B.G. 227 Sterling Hall of Medicine, New Haven, Conn.*

HUMMEL, KATHARINE P., Ph.D., Research Associate, *Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.*

HUMPHREY, RUFUS R., A.M., Ph.D., Professor of Anatomy, School of Medicine, University of Buffalo, *24 High St., Buffalo, N. Y.*

HUMPHREY, TRYPHENA, A.M., M.D., Ph.D., Professor of Anatomy, *University of Pittsburgh School of Medicine, Pittsburgh 13, Pa.*

HUNT, THOMAS E., A.B., Ph.D., Professor of Anatomy, *Medical College of Alabama, Birmingham 5, Ala.*

HUNTER, OSCAR B., A.B., A.M., M.D., Suite *1000, Columbia Med. Bldg. Annex, 915 — 19th St., N.W., Washington 6, D. C.*

HUTCHINSON, CRANFORD, Ph.D., Assistant Professor of Anatomy, *Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia 7, Pa.*

IFFT, JOHN D., A.B., M.A., Ph.D., Assistant Professor of Anatomy, *Boston University School of Medicine, 80 E. Concord Street, Boston 18, Massachusetts.*

INGERSOLL, EVERETT H., B.S., M.S., Ph.D., Associate Professor of Anatomy, *Medical College of Virginia, Richmond, Va.*

INGRAM, W. R., M.S., Ph.D., (Exec. Com. '48–), Professor and Head, Department of Anatomy, *State University of Iowa, College of Medicine, Iowa City, Ia.*

JACKSON, JOSEPH L., M.A., M.B., Professor of Anatomy, *University of Saskatchewan, Saskatoon, Canada.*

JAILER, JOSEPH WILLIAM, B.S., M.S., Ph.D., M.D., Associate in Medicine, *College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York 32, N. Y.*

JANES, RALPH G., A.B., B.S., M.S., Ph.D., Associate Professor of Anatomy, *Dept. of Anatomy, State University of Iowa, College of Medicine, Iowa City, Iowa.*

JANSEN, JAN, M.D., Professor of Anatomy, *Anatomical Institute, University of Oslo, Oslo, Norway.*

JAYNE, EDGAR P., B.A., M.S., Ph.D., Assistant Professor of Anatomy, *The George Washington University Medical School, 1335 H Street, N.W., Washington 5, D. C.*

JEFFERS, KATHARINE R., Ph.D., Dean of Women, *College of William and Mary, Williamsburg, Va.*

JENKINS, GEORGE B., M.D., Emeritus Professor of Anatomy and Director of Anatomy Department, *George Washington University Medical School, Washington, D. C.*

JENSEN, ARTHUR V., M.A., Ph.D., Professor of Biology, *Adelphi College, Garden City, New York.*

JOB, THESLE T., A.B., M.S., Ph.D., Chairman of Department of Anatomy and Assistant Dean, Professor of Anatomy, *Loyola University School of Medicine, 706 South Wolcott Ave., Chicago, Ill.*

JOBIN, PIERRE, M.D., Professor of Anatomy, *Laval University Medical School, Quebec City, P.Q., Canada.*

JOHNSON, DOROTHY DOLE, A.B., M.A., Ph.D., Instructor, *Department of Anatomy, College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York 32, N. Y.*

JOHNSON, MYRA L., A.B., A.M., Ph.D., M.D., Intern, *University Hospital, 2065 Adelbert Road, Cleveland, Ohio.*

JOHNSON, PERCY L., B.S., M.S., Ph.D., Associate Professor of Histology, *College of Dentistry, New York University, 477 First Avenue, New York 16, N. Y.*

JOHNSON, SYDNEY E., Ph.D., M.D., F.A.C.P., Professor of Radiology, School of Medicine, University of Louisville; *2800 Riedling Drive, Louisville 6, Ky.*

JOHNSTON, THOMAS BAILLIE, C.B.E., M.D., Emeritus Professor of Anatomy, University of London, *Guy's Hospital, London, England, S. E. 1.*

JOHNSTONE, PAUL N., A.B., M.D., *Suite 1110 Bryant Blvd., Kansas City. Mo.*

JONES, DAVID S., M.S., Ph.D., M.D., Associate Professor of Anatomy, *Stritch School of Medicine of Loyola University, 706 S. Wolcott St., Chicago, Ill.*

JONES, OLIVER P., A.B., Ph.D., Assistant Dean and Professor of Anatomy, *University of Buffalo, School of Medicine, 24 High St., Buffalo, N. Y.*

JONES, RUSSELL L., A.B., Ph.D., M.D., Interne, *Hollywood Presbyterian Hospital, Hollywood, Calif.*

JORDAN, HARVEY ERNEST, A.M., Ph.D., Sc.D., (Ex. Com. '18–'21) (First Vice-Pres. '36–'38), *34 University Circle, Charlottesville, Va.*

JOY, EDWARD A., A.B., A.M., Ph.D., Associate Professor of Anatomy, *Tufts College Medical School, 136 Harrison Ave., Boston 11, Mass.*

JUHN, MARY, Ph.D., Research Professor, Department of Poultry Husbandry, *University of Maryland, College Park, Maryland.*

JUMP, ELLIS B., D.M.D., Ph.D., Professor of Anatomy, *University of Oregon Dental School, Portland 1, Oregon.*

JUNQUEIRA, L. C. U., M.D., Associate Professor, Laboratorio de Histologia and Embriologia, *Caixa Postal 100B., S. Paulo, Brasil.*

KAAN, HELEN WARTON, A.B., Ph.D., Research Associate, *Chemical-Biological Coordination Center, National Research Council, 2101 Constitution Ave., Washington 25, D. C.*

KABAT, HERMAN, Ph.D., M.D., Medical Director, *Kabat-Kaiser Institute for Neuromuscular Rehabilitation; c/o Permanente Foundation Hospital, Oakland 11, Calif.*

KAISER, ROBERT A., A.B., M.D., Instructor in Anatomy, *School of Medicine, University of Buffalo, 24 High St., Buffalo, New York.*

KAMMERAAD, ADRIAN, A.B., Ph.D., Director of Research, Kremers-Urban Co., *1630 E. Newton Ave., Milwaukee 11, Wis.*

KAMPMEIER, OTTO FREDERIC, A.B., Ph.D., M.D., Professor of Anatomy and Head of Department, *College of Medicine, University of Illinois, 1853 W. Polk St., Chicago, Ill.*

KAPLAN, EMANUEL B., B.S., M.D., Assistant Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York 32, N. Y.*

KARCZMAR, ALEXANDER G., Ph.D., Associate Professor of Pharmacology, *Georgetown University School of Medicine, Washington 7, D. C.*

KATSH, GRACE F., B.A., M.S., Ph.D., Research Associate, *Brookhaven National Laboratories, Upton, Long Island, N. Y.*

KAYLOR, CORNELIUS T., A.B., M.S., Ph.D., Associate Professor of Anatomy, *School of Medicine, University of North Carolina, Chapel Hill, N. C.*

KEITH, SIR ARTHUR, M.D., LL.D., F.R.C.S., D.Sc., F.R.S., Master of *Buckston Browne Research Farm, Downe, Kent, England.*

KELLOGG, HOWARD B., *University of Washington, Anatomy Department, Seattle 5, Washington.*

KELLOGG, REMINGTON, Ph.D., Director, *U. S. National Museum, Washington 25, D. C.*

KELLY, G. LOMBARD, A.B., B.S., M.D., Dean and Professor of Anatomy, *University of Georgia, School of Medicine, Augusta, Ga.*

KELSALL, MARGARET ASTON, B.A., M.A., Ph.D., Research Associate, *Department of Biology, University of Colorado, Boulder, Colorado.*

KEY, J. ALBERT, B.S., M.D., Clinical Professor of Orthopedic Surgery, *Washington University, St. Louis, Mo.*

KILLE, FRANK R., Ph.D., B.S., M.S., Professor of Zoology and Dean of The College, *Carleton College, Northfield, Minn.*

KIMMEL, DONALD L., Ph.D., Professor of Anatomy, *School of Medicine, Temple University, Broad St. at Ontario, Philadelphia, Pa.*

KINDRED, JAMES ERNEST, A.B., A.M., Ph.D., Associate Professor of Anatomy, *University of Virginia Medical School, Box 1873, University Station, Charlottesville, Va.*

KING, HELEN DEAN, A.M., Ph.D., Member of The Wistar Institute (Experimental Zoology), *The Fairfax Apts., 43rd and Locust Sts., Philadelphia 4, Pa.*

KING, JESSIE L., Ph.D., Professor Emeritus, Goucher College, Baltimore, Md., *5 Maryland Ave., Towson 4, Md.*

KINGERY, HUGH MCMILLAN, A.B., A.M., Ph.D., Professor of Visual Education, *School of Medicine, University of Colorado, Denver, Colo.*

KINGSLEY, DANIEL M., M.S., Ph.D., M.D., Orthopaedic Surgeon, *530 DeSoto St., Alexandria, La.*

KIRGIS, HOMER D., M.D., Ph.D., Instructor in Anatomy, *Tulane University School of Medicine, New Orleans 15, Louisiana.*

KIRKHAM, WILLIAM BARRI, Ph.D., Consulting Director, Museum of Natural History, *220 State St., Springfield 5, Mass.*

KIRKMAN, HADLEY, A.B., S.M., Ph.D., Professor of Anatomy, *Stanford University, Calif.*

KIRSCHBAUM, ARTHUR, Ph.D., M.D., Professor and Head, Department of Anatomy, *College of Medicine, University of Illinois, 1853 West Polk St., Chicago 12, Illinois.*

KLAPPER, CLARENCE EDWARD, A.B., M.A., Ph.D., Assistant Professor of Anatomy, *Medical College of Alabama, 620 South 20th Street, Birmingham 5, Alabama.*

KNISELY, MELVIN H., Ph.D., Professor and Chairman, Anatomy Dept., *Medical College of the State of South Carolina, Charleston 16, S. C.*

KNOUFF, R. A., B.A., M.A., Ph.D., Professor of Anatomy, *Department of Anatomy, Ohio State University, Columbus, O.*

KOCH, JOHN C., B.S., M.D., *1053 Iroquois Ave., Detroit 14, Mich.*

KOENIG, HAROLD, B.S., B.M., M.D., M.S., Ph.D., Assistant Professor of Anatomy, *The Chicago Medical School, 710 South Wolcott St., Chicago, Ill.*

KOLOUCH, FRED, JR., A.B., M.A., M.B., M.D., Ph.D., Surgeon, *222 Seventh Ave., North Twin Falls, Idaho.*

KOLLROS, JERRY JOHN, B.S., Ph.D., Associate Professor of Zoology, *Department of Zoology, State University of Iowa, Iowa City, Ia.*

KONEFF, ALEXEI ALEXANDER, M.D., Assistant Professor of Anatomy and Lecturer in Histological Technique, *University of California Medical School, Life Sciences Building, Berkeley 4, Calif.*

KORNHAUSER, S. I., M.A., Ph.D., (Ex. Com. '42–'49), Chairman, Department of Anatomy, *University of Louisville, School of Medicine, 101 W. Chestnut St., Louisville 2, Ky.*

KRAEMER, DOROTHY ZIEGLER, B.A., M.A., Ph.D., Assistant Professor of Anatomy, *Long Island College of Medicine, 350 Henry St., Brooklyn 2, New York.*

KRAHL, VERNON E., B.S., M.S., Ph.D., Associate Professor of Gross Anatomy, *University of Maryland Medical School, 29 South Greene St., Baltimore 1, Md.*

KRAMER, THEODORE C., B.S., M.A., Ph.D., Assistant Professor of Anatomy, *University of Michigan, Ann Arbor, Mich.*

KREHBIEL, ROBERT H., M.S., Ph.D., Associate Professor of Anatomy, *University of Illinois, College of Medicine, 1853 W. Polk St., Chicago 12, Ill.*

KRIEG, WENDELL J. S., B.S., M.S., Ph.D., Professor of Anatomy, *Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill.*

KROPP, BENJAMIN, A.M., Ph.D., Associate Professor, Department of Anatomy, *Queen's University Medical School, Kingston, Ontario, Canada.*

KUHLENBECK, HARTWIG, M.D., Ph.D., Professor of Anatomy, *Woman's Medical College of Pennsylvania, Philadelphia, Pa.*

KUNKEL, BEVERLY WAUGH, Ph.B., Ph.D., Professor of Biology, *Lafayette College, Easton, Pa.*

KUNTZ, ALBERT, Ph.D., M.D. (Second Vice-Pres. '38–'39; First Vice-Pres. '39–'40), Professor of Anatomy and Director of the Department, *St. Louis University Medical School, 1402 South Grand Blvd., St. Louis, Mo.*

KUTCHIN, MRS. HARRIET LEHMANN, A.M., *"The Maplewood," Green Lake, Wis.*

LACHMAN, ERNEST, M.D., Professor of Anatomy, Chairman of the Department, *University of Oklahoma School of Medicine, Oklahoma City, Okla.*

LANGSTON, WILLIAM CLEAVER, A.B., M.S., M.D., Professor of Anatomy, Department Head, *School of Medicine, University of Arkansas, McAlmont St., Little Rock, Ark.*

LANGWORTHY, ORTHELLO R., A.B., A.M., M.D., Associate Professor of Psychiatry, Johns Hopkins University, *Johns Hopkins Hospital, Baltimore, Md.*

LANIER, RAYMOND R., Ph.D., M.D., Professor of Radiology, *University of Colorado Medical Center, 4200 East Ninth Ave., Denver 7, Colorado.*

LANSING, ALBERT I., Ph.D., Associate Professor of Anatomy, *Washington University, School of Medicine, St. Louis 10, Missouri.*

Larsell, Olof, M.D., Ph.D., (Ex. Com. '38–'42), Professor of Anatomy, *University of Oregon Medical School, Portland, Ore.*

Lasker, Gabriel Ward, A.B., M.A., Ph.D., Assistant Professor of Anatomy, *Wayne University College of Medicine, 1512 St. Antoine Street, Detroit 26, Mich.*

Lassek, Arthur M., M.S., Ph.D., M.D., Professor of Anatomy and Chairman of the Department, *Boston University School of Medicine, 80 E. Concord St., Boston 18, Mass.*

Latimer, Homer B., A.M., Ph.D., Professor of Anatomy, *Medical School, University of Kansas, Lawrence, Kan.*

Latta, John Stephens, A.B., Ph.D., Professor of Anatomy and Chairman of the Department, *University of Nebraska, College of Medicine, Forty-second St. and Dewey Ave., Omaha 5, Neb.*

Lauer, Edward W., B.S., M.A., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, E. Medical Bldg., University of Michigan, Ann Arbor, Michigan.*

Lawless, John J., M.A., Ph.D., Director, University Health Service, *West Virginia University, Morgantown, W. Va.*

Lazarow, Arnold, M.D., Ph.D., Associate Professor of Anatomy, *School of Medicine, Western Reserve University, 2109 Adelbert Rd., Cleveland 6, O.*

Leathem, James H., B.S., M.A., Ph.D., Professor of Zoology, *Rutgers University, New Brunswick, N. J.*

Leblond, Charles Philippe, M.D., Ph.D., Professor of Anatomy, *Department of Anatomy, McGill University, Montreal, P. Q., Canada.*

Leduc, Elizabeth Hortense, B.S., M.A., Ph.D., Instructor in Anatomy, *Harvard Medical School, 25 Shattuck St., Boston 15, Mass.*

Leonard, Samuel L., Ph.D., Associate Professor of Zoology, *Cornell University, Ithaca, N. Y.*

LeQuire, Virgil Shields, B.A., M.D., Assistant Professor of Anatomy, *Vanderbilt University Medical School, Nashville 4, Tenn.*

Levenstein, Irving M., Ph.D., Endocrinologist, *Leberco Laboratories, Bloomfield, N. J.*

Lev, Maurice, B.S., M.D., Associate Professor of Pathology, *University of Illinois, School of Medicine, 1853 West Polk St., Chicago 12, Ill.*

Levi, Giuseppe, M.D., Professor of Anatomy, Histology and Embryology, *Institute Anatomica, the University of Turin, Corte M. D'Areglia 52, Torina, Italy.*

Levi-Montalcini, Rita, M.D., Research Associate of the National Council of Research in the Anatomic Institute of Turin (Italy); *Department of Zoology, Washington University, St. Louis 5, Mo.*

Lewis, Frederic T., A.M., M.D., (Ex. Com. '09–'13, Vice-Pres. '14–'16; Pres. '36–'38), Professor of Comparative Anatomy, Emeritus, *Harvard Medical School, Boston 15, Mass.*

Lewis, Margaret Reed, *The Wistar Institute, 36th St. and Woodland Ave., Philadelphia 4, Pa.*

Lewis, Warren Harmon, B.S., M.D., (Ex. Com. '09–'11, '14–'17, Pres. '34–'36), *The Wistar Institute, 36th St. and Woodland Ave., Philadelphia 4, Pa.*

Lhotka, John Francis, Jr., B.A., M.S., B.Med., Interne, *Minneapolis General Hospital, Minneapolis 15, Minn.*

LIEDKE, KATHE B., A.B., M.Sc., Ph.D., Lecturer, *Hunter College, New York; 453 Warwick Ave., West Englewood, New Jersey.*

LIMSON, MARCIANO, M.D., Professor and Head, Department of Anatomy, *College of Medicine, University of the Philippines, Manila, P. I.*

LINELL, ERIC AMBROSE, Ch.B., M.D., Professor of Neuropathology, *Banting Institute, University of Toronto, Canada.*

LIPSHUTZ, BENJAMIN, M.D., Assistant Professor in Neuro-anatomy, Jefferson Medical College, *1007 Spruce St., Philadelphia, Pa.*

LITTLE, CLARENCE COOK, B.A., M.S., Sc.D., LL.D., Litt.D., Director, *Jackson Memorial Laboratory, Bar Harbor, Me.*

LIU, CHAN-NAO, B.S., Ph.D., Associate in Anatomy, *University of Pennsylvania, School of Medicine, Philadelphia 4, Pa.*

LLOYD, RUTH SMITH, A.B., M.S., Ph.D., Instructor in Anatomy, *Howard University, Washington, D. C.*

LOBO, BRUNO ALIPIO, B.A., M.D., ''Docente Livre'' Professor of Histology and Embryology, *Faculdade Nacional de Medicina Universidade do Brasil, Avenida Pasteur No. 458, Praia Vermelha, Rio de Janeiro, Brasil.*

LONG, MARGARET ELEANOR, B.S., M.S., Ph.D., Assistant Professor of Biology, *Dept. of Obstetrics and Gynecology, Columbia University, College of Physicians and Surgeons, 630 W. 168th St., New York 32, N. Y.*

LOOSLI, CLAYTON G., M.D., Ph.D., Professor of Preventive Medicine, *Department of Medicine, University of Chicago, 950 E. 59th St., Chicago 37, Ill.*

LORD, FREDERIC P., A.B., M.D., Professor of Anatomy, *Dartmouth Medical School, 39 College St., Hanover, N. H.*

LORENTE DE NÓ, RAPHAEL, M.D., Member, *Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City 21.*

LOW, FRANK NORMAN, A.B., Ph.D., Associate Professor of Anatomy, *Louisiana State University School of Medicine, New Orleans 13, La.*

LOWRANCE, EDWARD WALTON, A.B., M.A., Ph.D., Associate Professor of Anatomy, *School of Medicine, University of Missouri, Columbia, Missouri.*

LOWREY, LAWSON GENTRY, A.M., M.D., Assistant Clinical Professor of Psychiatry, Columbia University College of Physicians and Surgeons, Editor, *American Journal Orthopsychiatry, 25 W. 54th St., New York 19, N. Y.*

LUCAS, ALFRED M., Ph.D., Cytopathologist, *U. S. Regional Poultry Research Laboratory, E. Mt. Hope Rd., East Lansing, Mich.*

LUCAS, MIRIAM SCOTT, Ph.D., (Mrs. A. M.), Assistant Professor Biological Science, *Michigan State College, East Lansing, Mich.*

LYONS, WILLIAM R., B.A., M.A., Ph.D., Professor of Anatomy, *University of California, Berkeley 4, Calif.*

MACCALLUM, DANIEL BARTLETT, S.B., Ph.D., M.D., *Veterans Administration Center, Los Angeles 25, Calif.*

MACCARDLE, ROSS C., B.S., Ph.D., Principal Cytologist, *National Cancer Institute, Bethesda 14, Md.*

MACFALL, CLAUDE M., Ph.D., *Department of Anatomy, Meharry Medical College, Nashville 8, Tenn.*

MACFARLAND, WILLIAM ELLERY, B.A., Ph.D., Editor, *W. B. Saunders and Company, Seventh and Locust Streets, Philadelphia, Pa.*

Macklin, Charles C., M.B., M.D., M.A., Ph.D., F.R.S.C. (Ex. Com. '28–'32), Research Professor of Histology, *University of Western Ontario Medical School, 346 South St., London, Ontario, Canada.*

Macklin, Madge Thurlow, A.B., M.D., LL.D., Research Associate in Medicine; Lecturer in Medical Genetics, *B and Z Building, Ohio State University, Columbus, Ohio.*

MacLeod, John, A.B., M.S., Ph.D., Assistant Professor of Anatomy, *Cornell University Medical College, 1300 York Ave., New York City.*

Magath, Thomas Byrd, M.Sc., Ph.D., Ph.B., M.D., Chief, Division Clinical Laboratories, Mayo Clinic, and Professor Pathology and Parasitology, *University of Minnesota, Mayo Clinic, Rochester, Minn.*

Magoun, Horace W., Ph.D., Professor and Chairman Department of Anatomy, *The Medical School, University of California, Los Angeles 24, California.*

Magruder, Samuel R., B.S., M.A., Ph.D., Associate Professor of Anatomy, *Tufts College Medical School, 136 Harrison Ave., Boston 11, Mass.*

Mahon, Eleanor Conway, Ph.D., *Iron River, Mich.*

Mainland, Donald, M.B., Ch.B., D.Sc., F.R.S.E., F.R.S.C., Professor of Biostatistics, *Department of Preventive Medicine, New York University - Bellevue Medical Center, 477 First Avenue, New York 16, N. Y.*

Malone, Edward F., A.B., M.D., (Ex. Com. '31–'35), Francis Brunning Professor of Anatomy, *153 Cortland Ave., Winter Park, Florida.*

Manter, John T., A.B., Ph.D., Associate Professor of Microscopic Anatomy, *University of Georgia, School of Medicine, Augusta, Ga.*

Markee, J. E., B.S., Ph.D., Professor of Anatomy, Head of Anatomy Department, *Duke University School of Medicine, Durham, N. C.*

Martin, Cecil P., M.A., M.B., D.Sc., Professor of Anatomy, *McGill University, Montreal, Canada.*

Martin, John, B.S., M.S., M.B., M.D., Ph.D., Associate Professor of Surgery, *Northwestern University Medical School, 700 N. Michigan Ave., Chicago, Ill.*

Marvin, Horace Newell, B.A., M.A., Ph.D., Associate Professor of Anatomy, *University of Arkansas School of Medicine, Little Rock, Arkansas.*

Mason, Karl E., Ph.D., D.Sc., Professor of Anatomy, *The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*

Masson, Georges M. C., D.V.M., Ph.D., *Research Division, Cleveland Clinic Foundation, Cleveland 6, Ohio.*

Mastin, E. Vernon, M.D., M.S. (in Surgery), Assistant Professor of Surgery, St. Louis University Medical School, *3720 Washington Ave., St. Louis, Mo.*

Matas, Rudolph, M.D., LL.D., Sc.D., F.R.C.S. Eng. (hon.), (Charter member), Professor of Surgery (1894–1927), Emeritus (1927–), and Demonstrator of Anatomy (1885–1894), Tulane University, *2255 St. Charles Ave., New Orleans 13, La.*

Matthews, Samuel A., M.A., Ph.D., Professor of Biology, *Williams College, Williamstown, Mass.*

Mavor, James Watt, B.A., M.A., Ph.D., Professor of Biology, *8 Gracewood Park, Cambridge, Mass.*

Mayer, Edmund, M.D., Pathologist, Chemotherapy Division, Stamford Research Laboratories, *American Cyanamid Company, 1937 W. Main Street, Stamford, Conn.*

McClendon, Jesse F., Ph.D., Research Professor of Physiology, Hahnemann Medical College, *Route 1, Trooper Rd., Norristown, Pa.*

McClure, Charles Freeman Williams, A.M., Sc.D. (Vice-Pres. '10–'11, Ex. Com. '12–'16, Pres. '20–'21), Professor Emeritus of Comparative Anatomy, *Princeton University, 1 Battle Road, Princeton, N. J.*

McCotter, Rollo E., M.D., Professor of Anatomy, Medical School, University of Michigan, *Lakewood R. R. 1, Ann Arbor, Mich.*

McCrady, Edward, Jr., A.B., M.S., Ph.D., Professor of Biology, *Biology Division, Atomic Energy Com., Oak Ridge, Tenn.*

McCulloch, Warren S., A.B., A.M., M.D., Associate Professor of Psychiatry, College of Medicine, University of Illinois, *912 South Wood St., Chicago, Ill.*

McCullough, Albert William, A.B., B.Sc., M.A., Ph.D., Associate Professor of Anatomy, *University of Arkansas School of Medicine, Little Rock, Arkansas.*

McKenzie, Fred F., B.S.A., A.M., Ph.D., D.Agr., D.V.S., Professor of Animal Husbandry and Chairman of Department, *Oregon State College, Corvallis, Ore.*

McKinney, Roscoe L., Ph.D., Professor of Anatomy, *Howard University, Washington, D. C.*

McNaughton, Francis L., B.A., M.Sc., M.D., Assistant Professor of Neurology, *School of Medicine, McGill University, 3801 University Street, Montreal 2, Canada.*

McNutt, C. W., A.B., M.S., Ph.D., Assistant Professor, *Department of Anatomy, The University of Texas, Galveston, Texas.*

McVay, Chester Bidwell, M.D., Ph.D., Clinical Professor of Surgery, *University of South Dakota, Yankton, South Dakota.*

Mead, Harold T., Ph.D., Professor of Biology, *Rider College, Trenton 9, N. J.*

Meader, Ralph G., A.B., A.M., Ph.D., Chief, Cancer Research Grants Branch, National Cancer Institute; *200 Chandler St., Maplewood, Bethesda, Md.*

Meaker, Samuel R., A.B., A.M., M.D., M.R.C.S. (Eng.), F.A.C.S., F.C.O.G., Professor of Gynaecology, Boston University School of Medicine, *475 Commonwealth Ave., Boston, Mass.*

Mendelsohn, William, M.S., M.D., Clinical Instructor in Surgery, Yale Medical School, *442 Temple St., New Haven, Conn.*

Menkin, Valy, B.S., M.A., M.D., Associate Professor of Experimental Pathology, Department of Surgical Research, *Temple University Medical School, Broad Street at Ontario, Philadelphia 40, Pa.*

Menschik, Zygmunt, B.A., M.B., Ch.B., M.D., Assistant Professor of Anatomy, *School of Medicine, University of Ottawa, Ottawa, Canada.*

Merriman, Daniel, B.S., M.S., Ph.D., Associate Professor of Biology and Director, Bingham Oceanographic Laboratory, *Box 2025, Yale Station, Yale University, New Haven, Conn.*

Mettler, Fred. A., A.M., M.D., Ph.D., Professor of Anatomy, College of Physicians and Surgeons, Columbia University, *630 West 168th St., New York City 32.*

Meyer, Arthur W., S.B., M.D., (Ex. Com. '12–'16, Vice-Pres. '24–'25), Professor of Anatomy Emeritus, Leland Stanford Junior University, *121 Waverly St., Palo Alto, Calif.*

Meyer, Roland K., B.A., M.A., Ph.D., Professor of Zoology, *Department of Zoology, University of Wisconsin, Madison, Wis.*

Michels, Nicholas A., M.A., D.Sc., Professor of Anatomy, *The Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia, Pa.*

Midlo, Charles, M.D., Instructor in Neurology, Lecturer in Hygiene, *Tulane University, 1404 Louisiana Ave., New Orleans 15, La.*

Miles, Meryl, B.S., M.S., Assistant Professor of Anatomy, *University of Wisconsin, Science Hall, Madison 6, Wis.*

Miller, James A., Jr., A.B., Ph.D., Professor and Chairman of Department of Anatomy, *Emory University School of Dentistry, Basic Science Building, Emory University, Georgia.*

Miller, Richard A., B.A., M.S., Ph.D., Assistant Professor, *Department of Anatomy, Albany Medical College, Albany, N. Y.*

Miller, Roland E., B.S., M.S., Ph.D., *361 E. 6th St., Erie, Pa.*

Miller, Ruth Alexander, A.B., Ph.D., Assistant Professor of Anatomy, Anatomy Department, *Louisiana State University, Medical School, New Orleans 13, La.*

Miller, Ruth N., A.B., M.D., *7703 West Chester Pike, Upper Darby, Pa.*

Miller, Shirley P., A.B., A.M., Ph.D., Assistant Professor of Anatomy, *Anatomical Institute, University of Minnesota, Minneapolis, Minn.*

Minckler, Jeff, Ph.D., M.D., Pathologist, *5404 S.E. Lake Rd., Milwaukie 22, Oregon.*

Monie, Ian Whitelaw, Assistant Professor of Anatomy, Medical College, University of Manitoba, *Bannatyne and Emily, Winnipeg, Manitoba, Canada.*

Montagna, William, A.B., Ph.D., Associate Professor of Biology, *Arnold Laboratory, Brown University, Providence 12, R. I.*

Montagu, M. F. Ashley, Ph.D., Chairman and Professor of Anthropology, *Rutgers University, Box 621, New Brunswick, New Jersey.*

Moore, Carl R., A.M., Ph.D., Professor of Zoology, *Hull Zoological Laboratory, The University of Chicago, Chicago, Ill.*

Morgan, Charles F., A.B., Ph.D., Professor of Physiology, *Georgetown University School of Medicine, Washington 7, D. C.*

Morgan, Lawrence Onis, A.B., A.M., Ph.D., Professor of Anatomy, *Indiana University Medical School, Bloomington, Indiana.*

Morin, Ferdinando, B.A., M.D., Ph.D., Instructor in Anatomy, *Wayne University College of Medicine, 1512 St. Antoine St., Detroit 26, Michigan.*

Morison, Robert S., A.B., M.D., Assistant Director for the Medical Sciences, *The Rockefeller Foundation, 49 West 49th St., New York 20, N. Y.*

Morrill, Charles V., A.M., Ph.D., Associate Professor of Anatomy, *Cornell University Medical College, 1300 York Ave., New York City.*

Mortensen, Otto A., B.S., M.S., M.D., Professor of Anatomy, Assistant Dean of Medical School, *University of Wisconsin, 401 Science Hall, Madison 5, Wis.*

Morton, Dudley Joy, M.D., Associate Clinical Professor, Department of Anatomy, *College of Physicians and Surgeons, Columbia University, 14 E. 90th St., New York City.*

Morton, Thomas H., Ph.D., Chairman, Department of Biology, *Niagara University, Niagara University, N. Y.*

Mossman, Harland W., Ph.D., Associate Professor of Anatomy, *Science Hall, University of Wisconsin, Madison, Wis.*

Moyer, Elizabeth K., A.B., M.A., Ph.D., Assistant Professor of Anatomy, *Boston University Medical School, 80 E. Concord St., Boston 18, Mass.*

MURRAY, MARGARET RANSONE, A.B., M.S., Ph.D., Assistant Professor of Surgery, *Columbia University, College of Physicians and Surgeons, 630 West 168th Street, New York 32, N. Y.*

MURRAY, RAYMOND G., B.S., Ph.D., Associate Professor of Anatomy, *Indiana University Medical School, Bloomington, Indiana.*

MYERS, J. ARTHUR, M.S., Ph.D., M.D., Professor of Preventive Medicine and Chief of Chest Clinic, *University of Minnesota Medical School, Minneapolis, Minn.*

NELSON, EDWARD M., B.A., M.A., Ph.D., Associate in Anatomy, *Stritch School of Medicine, Loyola University, 706 S. Wolcott, Chicago 12, Illinois.*

NELSON, ROSE C., B.A., M.A., Ph.D., *37 DeVries Ave., North Tarrytown, N. Y.*

NELSON, WARREN O., A.B., Sc.M., Ph.D., *Department of Anatomy, State University of Iowa, College of Medicine, Iowa City, Iowa.*

NICHOLAS, JOHN SPANGLER, M.S., Ph.D., (Exec. Com. '47–), Sterling Professor of Biology, *Osborn Zoological Laboratory, Yale University, New Haven, Conn.*

NICHOLS, RAY JANNEY, A.B., Ph.D., Professor of Microscopic Anatomy, *School of Medicine, University of Mississippi, Box 124, University, Mississippi.*

NICOLL, PAUL ANDREW, A.B., Ph.D., Associate Professor of Physiology, *Indiana University, Bloomington, Indiana.*

NIELSON, PAUL E., M.S., Ph.D., *College of Medicine, University of Illinois, 912 South Wood St., Chicago 12, Ill.*

NIEMER, WILLIAM T., M.Sc., Ph.D., Assistant Professor of Anatomy, *The Creighton University Medical School, 302 North 14th Street, Omaha 2, Nebraska.*

NIGRELLI, ROSS F., B.S., M.S., Ph.D., Pathologist, New York Aquarium, New York Zoologist Society, Adjunct Associate Professor, New York University, *Bronx Zoo, Bronx Park, New York 60, N. Y.*

NOBACK, CHARLES ROBERT, M.S., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*

NOBACK, GUSTAVE J., B.S., M.A., Ph.D., Professor of Anatomy, Head of Department of Anatomy, *University of Puerto Rico School of Medicine, San Juan 22, Porto Rico.*

NOER, RUDOLPH J., A.B., M.D., Associate Professor of Surgery, Professor of Applied Anatomy, *Wayne University College of Medicine, 1512 St. Antoine St., Detroit 26, Michigan.*

NOWINSKI, WIKTOR W., Ph.D., Associate Professor of Neuro-Chemistry, Research Associate Tissue Culture Laboratory, *University of Texas Medical Branch, Galveston, Texas.*

OBENCHAIN, JEANETTE B., Ph.D., Research Associate, Department of Anatomy, *The University of Chicago, Chicago, Ill.*

ODIORNE, JOSEPH M., B.S., M.A., Ph.D., Assistant Professor of Anatomy, *New York University College of Medicine, 477 First Ave., New York 16, N. Y.*

O'DONOGHUE, CHARLES H., D.Sc., F.Z.S., F.R.S.E., Professor of Zoology, *University of Reading, Reading, England.*

O'LEARY, JAMES L., Ph.D., M.D., Associate Professor of Neuroanatomy and Assistant Professor of Neurology, *Washington University School of Medicine, St. Louis, Mo.*

OLSEN, MARLOW WILLIAM, B.S., M.S., Ph.D., Physiologist, *Bureau of Animal Industry, U. S. Department of Agriculture. Beltsville, Maryland.*

OLSZEWSKI, JERZY, M.D., Assistant Professor of Neuroanatomy, *McGill University and Montreal Neurological Institute, 3801 University St., Montreal 2, Canada.*

OPPENHEIMER, JANE M., Ph.D., Associate Professor of Biology, *Bryn Mawr College, Bryn Mawr, Pa.*

O'RAHILLY, RONAN, M.B., B.Ch., B.A.O., Ainsworth Scholar in Anatomy, National University of Ireland; Visiting Associate Professor of Anatomy, *Wayne University College of Medicine, 1512 St. Antoine St., Detroit 26, Mich.*

ORSINI, MARGARET WARD, B.A., Ph.D., Public Health Post-doctoral Research Fellow, *Department of Anatomy, Science Hall, University of Wisconsin, Madison, Wis.*

OSBORN, CLINTON M., B.A., M.A., Ph.D., Professor of Zoology and Director of Radioisotope Laboratory, *Syracuse University, Syracuse, N. Y.*

OSTERUD, HJALMER L., A.B., A.M., Ph.D., Professor of Anatomy, *Medical College of Virginia, Richmond, Va.*

OVERHOLSER, MILTON D., A.B., A.M., Ph.D., M.D., Professor of Anatomy and Chairman of Department, *University of Missouri, Columbia, Mo.*

PAFF, GEORGE H., M.A., Ph.D., Professor of Anatomy, *Hahnemann Medical College, 235 N. 15th St., Philadelphia 2, Pa.*

PALADE, GEORGE EMIL, M.D., Fellow at the Rockefeller Institute, *Rockefeller Institute for Medical Research, York Ave. and 66th St., New York, N. Y.*

PALAY, SANFORD L., A.B., M.D., Assistant Professor of Anatomy, *Yale University School of Medicine, 333 Cedar St., New Haven, 11, Conn.*

PALMER, CARROLL E., M.A., Ph.D., M.D., Senior Surgeon, *Field Studies Office, Tuberculosis Control Division, U. S. Public Health Service, Washington 25, D. C.*

PALMER, DWIGHT M., B.Sc., M.Sc., M.D., Professor and Chairman, Department of Neurology and Psychiatry, Professor of Anatomy, Ohio State University; *University Hospital, Ohio State University, Columbus 10, Ohio.*

PANKRATZ, DAVID S., A.B., A.M., Ph.D., M.D., Dean of the University of Mississippi School of Medicine and Professor of Anatomy, *University of Mississippi, Box 15, University, Miss.*

PAPANICOLAOU, GEORGE N., Ph.D., M.D., Professor of Clinical Anatomy, *Cornell University Medical College, 1300 York Ave., New York City.*

PAPEZ, JAMES WENCESLAS, B.A., M.D., Professor Emeritus, Cornell University, Director of Research, Mental Hygiene, Dept. of Public Welfare, Ohio, *Columbus State Hospital, 1960 West Broad St., Columbus, Ohio.*

PARKER, GEORGE HOWARD, D.Sc., Professor of Zoology, Emeritus, *Harvard University, 16 Berkeley St., Cambridge 38, Mass.*

PARKER, RAYMOND C., Ph.D., Associate Professor of Experimental Cytology, School of Hygiene, and Research Associate, Connaught Medical Research Laboratories, *University of Toronto, Toronto, Canada.*

PARKES, A. S., D. S., F.R.S., Member, *National Institute for Medical Research, Hampstead, London, N.W. 3, England.*

PARNELL, JEROME PATRICK, B.S., M.S., Ph.D., Assistant Professor, *State University Medical Center at N.Y.C., Brooklyn, N. Y.*

PARSHLEY, MARY STEARNS, Ph.D., Research Associate, Department of Surgery, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York 32, N. Y.*

PATEK, PAUL R., B.A., Ph.D., Professor of Anatomy and Head of the Department, *School of Medicine, University of Southern California, Los Angeles 7, Calif.*

PATTEN, BRADLEY MERRILL, A.M., Ph.D. (Second Vice-Pres. '34–'36), Professor and Chairman, Department of Anatomy, *University of Michigan Medical School, 2500 E. Medical Bldg., Ann Arbor, Mich.*

PATTERSON, JOHN THOMAS, B.S., Ph.D., Professor of Zoology, *University of Texas, Austin, Tex.*

PATTERSON, JOHN W., A.B., M.S., Ph.D., M.D., Assistant Professor of Anatomy, *School of Medicine, Western Reserve University, Cleveland 6, Ohio.*

PEARSON, ANTHONY A., M.A., Ph.D., Professor of Anatomy, *School of Medicine, University of Oregon, Portland, Oregon.*

PEDERSEN, HERBERT EUGENE, A.B., M.D., Instructor in Surgery, *Wayne University College of Medicine, Detroit, Mich.*

PEELE, TALMAGE LEE, A.B., M.D., Associate Professor of Anatomy, Associate in Medicine, *Duke Hospital, Box 3811, Durham, N. C.*

PEIRCE, EDMUND CONVERSE, 2ND, S.B., M.D., Fellow in Cardiovascular Surgery, N.I.H., Baltimore Marine Hospital, *4512 Marble Hall Road, Baltimore 12, Md.*

PENCHARZ, RICHARD I., Ph.D., Chief of Research, Laboratory for Exp. Biology, *Stanford Research Inst., Stanford, Calif.*

PERKINS, ORMAN C., A.B., A.M., M.D., F.A.C.P., Professor of Neurology, Long Island College of Medicine, *829 Carroll St., Brooklyn, N. Y.*

PFEIFFER, CARROLL A., Ph.D., Assistant Professor of Anatomy, *Yale University School of Medicine, 333 Cedar St., New Haven, Conn.*

PHELPS, DORIS H., A.B., M.A., Ph.D., Research Associate in Gynecology and Obstetrics, *Vanderbilt University School of Medicine, Nashville, Tenn.*

PHILLIPS, HOWARD MITCHELL, B.S., M.A., Ph.D., Professor of Biology, *Emory University, Georgia.*

PIATT, JEAN, B.S., M.A., Ph.D., Associate Professor of Anatomy, *School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*

PICK, JOSEPH, M.D., Associate Professor of Anatomy, *New York University, College of Medicine, 477 First Ave., New York City.*

PICKFORD, GRACE E., Ph.D., Research Associate, *Bingham Oceanographic Laboratory, Yale University, Box 2025, Yale Station, New Haven, Connecticut.*

PIERSOL, WILLIAM HUNTER, B.A., M.B., *103 Hudson Drive, Toronto, Canada.*

PINCUS, GREGORY, B.S., M.S., Sc.D., Director of Laboratories, Worcester Foundation for Experimental Biology, *222 Maple Ave., Shrewsbury, Mass.*

PLAGGE, JAMES C., S.B., Ph.D., Associate Professor of Anatomy, Department of Anatomy, College of Medicine, University of Illinois, *1853 W. Polk St., Chicago 12, Ill.*

PLISKE, EDWARD C., Ph.D., Assistant Professor of Anatomy, *Medical School, University of Michigan, Room 1014 East Medical Bldg., Ann Arbor, Mich.*

POLLISTER, ARTHUR WAGG, A.B., A.M., Ph.D., Associate Professor of Zoology, *Columbia University, New York 27, N. Y.*

POLYAK, STEPHEN, M.D., Professor of Anatomy, Department of Anatomy, *The University of Chicago, Chicago, Ill.*

POMERAT, CHARLES M., A.B., A.M., Ph.D., Professor of Anatomy, *University of Texas, Medical Branch, Galveston, Tex.*

POMERAT, GERARD ROLAND, A.B., A.M., Ph.D., Assistant Director for Natural Sciences, *Rockefeller Foundation, 49 W. 49th St., New York 20, N. Y.*

PORTER, KEITH ROBERTS, B.Sc., A.M., Ph.D., Associate, *Rockefeller Institute, 66th Street and York Avenue, New York 21, N. Y.*

POWER, RICHARD M. H., M.Sc., M.D.C.M., F.A.C.S., M.D., D.Sc., Clinical Resident, Gynecology and Obstetrics, St. Mary's Hospital; Session Lecturer, Associate in Research, Department of Anatomy, McGill University, Montreal, *1610 Sherbrooke St. West. Montreal, Quebec.*

POYNTER, CHARLES W. M., B.S., M.D., (Ex. Com. '19–'22), Professor of Anatomy, and Dean of the College of Medicine, University of Nebraska, *Forty-second and Dewey Ave., Omaha, Neb.*

PRATT, JEAN PAUL, M.D., Surgeon in Charge of Divisions of Gyñecology and Obstetrics, *Henry Ford Hospital, Detroit, Mich.*

PRYOR, JOSEPH WILLIAM, M.D., Emeritus Professor of Anatomy and Physiology, University of Kentucky, *417 West Second St., Lexington, Ky.*

PUCKETT, WILLIAM O., M.A., Ph.D., Professor and Chairman, *Department of Biology, Davidson College, Davidson, N. C.*

QUIRING, D. P., M.A., Ph.D., Associate Professor of Biology, Western Reserve University; Head of Anatomy Division, Cleveland Clinic Foundation, *2249 Demington Dr., Cleveland Heights, O.*

RALPH, PAUL H., B.A., M.S., Ph.D., Assistant Professor, *Department of Anatomy, University of Washington School of Medicine, Seattle 5, Wash.*

RAMSAY, ANDREW J., A.B., Ph.D., Professor of Histology and Embryology, *Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia 7, Pa.*

RAMSEY, ELIZABETH M., A.B., M.D., Research Associate, Carnegie Institution of Washington, Department of Embryology, Wolfe and Madison Streets, Baltimore 5, Md.; *3420 Que Street, N.W., Washington 7, D. C.*

RASMUSSEN, ANDREW T., Ph.D., (Ex. Com. '34–'38, 2nd Vice-Pres. '48–'50), Professor of Neurology, *Institute of Anatomy, University of Minnesota, Minneapolis, Minn.*

RASMUSSEN, GRANT LITSTER, Ph.D., Associate Professor of Anatomy, *University of Buffalo School of Medicine, 24 High Street, Buffalo, N. Y.*

RAWLES, MARY E., A.B., S.M., Ph.D., Research Associate (Embryology), *Department of Biology, Johns Hopkins University, Baltimore 18, Md.*

RAWLINSON, HERBERT E., M.D., M.Sc., Ph.D., Professor of Anatomy, *University of Alberta, Edmonton, Alberta, Canada.*

RAY, ROBERT D., M.D., Ph.D., Assistant Professor, Orthopedic Surgery, *Department of Surgery, School of Medicine, University of Washington, Seattle 5, Washington.*

REAGAN, FRANKLIN P., Ph.D., Lecturer in Anatomy, Hospitals Centre, Medical School, *The University, Edmund St., Birmingham, England.*

REBUCK, JOHN WALTER, B.A., M.A., M.D., Ph.D., Associate in Hematology, *Department of Laboratories, The Henry Ford Hospital, Detroit 2, Mich.*

REECE, RALPH P., B.Sc., M.Sc., Ph.D., Associate Professor and Associate Research Specialist in Dairy Husbandry, *New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, N. J.*

REED, ADRIAN F., A.B., M.A., Ph.D., M.D., Associate Professor of Anatomy, *Department of Anatomy, Tulane University Medical School, New Orleans 18, La.*

REESE, JOHN D., A.B., M.A., M.D., *North Carolina Sanatorium for the Treatment of Tuberculosis, McCain, North Carolina.*

REHMAN, IRVING, Ph.D., Associate Professor of Anatomy, *Department of Anatomy, School of Medicine, University of Southern California, Los Angeles 7, Calif.*

REINHARDT, WILLIAM O., A.B., M.D., Associate Professor of Anatomy, *University of California, Berkeley 4, Calif.*

REVELL, DANIEL GRAISBERRY, A.B., M.B., Professor Emeritus of Anatomy, University of Alberta, *9105 112 St., South Edmonton, Alberta, Canada.*

REYER, RANDALL WILLIAM, B.A., M.A., Ph.D., Assistant Professor of Anatomy, *University of Pittsburgh School of Medicine, Pittsburgh, 13, Pa.*

REYNOLDS, SAMUEL R. M., M.A., Ph.D., Sc.D., Dr. hon. causa, Staff Member, Physiologist and Lecturer in Obstetrics, Johns Hopkins University, *Department of Embryology, Carnegie Institution of Washington, Wolfe and Madison Sts., Baltimore, Md.*

RHINEHART, D. A., A.M., M.D., Professor of Applied Anatomy, University of Arkansas, *7200 W. Markham, Little Rock, Ark.*

RHINES, RUTH, B.S., M.S., Ph.D., M.D., Assistant Professor of Anatomy and Instructor in Medicine, *The School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*

RICE, EDWARD LORANUS, A.B., Ph.D., Sc.D., Professor Emeritus of Zoölogy, *Ohio Wesleyan University, 265 N. Sandusky St., Delaware, O.*

RICHARDS, OSCAR W., B.A., M.A., Ph.D., Research Supervisor for Biology, *American Optical Company, Research Laboratory, P.O. Box 137, Stamford, Conn.*

RICHARDSON, DOROTHY, A.B., Ph.D., Associate Professor of Zoology, *Connecticut College, New London, Conn.*

RICHINS, CALVIN A., B.A., M.A., Ph.D., Assistant Professor of Anatomy, *St. Louis University, School of Medicine, 1402 South Grand Boulevard, St. Louis 4, Missouri.*

RICHTER, KENNETH M., B.A., M.A., Ph.D., Associate Professor of Histology and Embryology, *University of Oklahoma, School of Medicine, Oklahoma City 4, Okla.*

RIENHOFF, WILLIAM F., JR., A.B., M.D., Associate Professor of Surgery, Johns Hopkins Hospital, *1201 North Calvert St., Baltimore, Md.*

RILEY, HENRY A., B.A., A.M., M.D., Professor of Clinical Neurology, *College of Physicians and Surgeons, Columbia University, 117 East 72nd St., New York City 21, N. Y.*

RING, JOHN ROBERT, A.B., ScM., Ph.D., Associate Professor of Anatomy, *Washington University School of Dentistry, 4559 Scott St., St. Louis 10, Mo.*

RIOCH, DAVID McK., M.D., Director of Research, Chestnut Lodge Sanitarium. *500 W. Montgomery Ave., Rockville, Md.*

RISLEY, PAUL L., B.A., M.S., Ph.D., Professor and Head of Biology Department. *University of Oregon, Eugene, Oregon.*

ROBERTS, JOSEPH THOMAS, B.A., M.S., M.D., Ph.D., F.A.C.P., C.V., Lecturer in Medicine, University of Buffalo School of Medicine, Chief Medical Staff, *Veterans' Administration Hospital, Buffalo, N. Y.*

ROBERTSON, G. GORDON, B.A., B.Sc., Ph.D., Associate Professor of Anatomy, *Baylor University, College of Medicine, Houston 1, Texas.*

ROBINSON, BYRON L., A.B., M.A., M.D., Associate Professor of Anatomy, *Chicago Medical School, 384 South York Street, Elmhurst, Illinois.*

ROGERS, JAMES B., A.M., M.D., Professor of Anatomy, *University of Louisville, School of Medicine, 101 W. Chestnut, Louisville, Ky.*

ROGERS, PHILIP V., Ph.D., Associate Professor of Biology, *Hamilton College, Clinton, N. Y.*

ROGERS, WILLIAM MITCHELL, B.S., Ph.D., Assistant Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 632 West 168th St., New York City.*

ROLLHÄUSER, HEINZ, M.D., *Department of Anatomy, University of Marburg/Lahn, Germany.*

ROLLHÄUSER, JOHANNA TER HORST, Ph.D., Assistant, *Department of Anatomy, University of Marburg/Lahn, Germany.*

ROMANES, GEORGE JOHN, B.A., Ph.D., M.B., Ch.B., Lecturer in Neuroanatomy, *Anatomy Department, University of Edinburgh, Teviot Place, Edinburgh 8, Scotland.*

ROMER, ALFRED S., B.A., Ph.D., Alexander Agassiz Professor of Zoology and Director of *Museum of Comparative Zoology, Harvard University, Cambridge 38, Mass.*

RONCORONI, ENRIQUE J., M.D., Professor of Anatomy, *Universidad del Litoral Rosario, Republic of Argentina.*

RONSTROM, G. N., B.S., M.S., M.D., Associate Professor of Anatomy, *Louisiana State University Medical Center, 1542 Tulane Ave., New Orleans, La.*

ROOFE, PAUL GIBBONS, B.S., Ph.D., Professor of Anatomy, *University of Kansas School of Medicine, Department of Anatomy, Lawrence, Kansas.*

ROOSEN-RUNGE, EDWARD C., M.D., Assistant Professor of Anatomy, *University of Louisville, School of Medicine, 101 W. Chestnut St., Louisville, Ky.*

ROSE, JERZY EDWIN, M.D., Assistant Professor of Physiology and Psychiatry, *Department of Physiology, Johns Hopkins Medical School, 710 N. Washington St., Baltimore 5, Md.*

ROSE, S. MERYL, B.A., M.A., Ph.D., Associate Professor of Zoology, *Department of Zoology, University of Illinois, Urbana, Ill.*

ROSSMAN, ISADORE, A.B., Ph.D., M.D., *113 W. 96th St., New York 25, N. Y.*

ROWLAND, LEWIS P., M.D., Assistant in Medicine, *Neurological Institute, 710 W. 168th St., New York City 32, N. Y.*

RUBINSTEIN, HYMAN S., Ph.G., B.S., M.D., Ph.D., Director of the Alfred Ullman Laboratory for Neuro-psychiatric Research, Sinai Hosp., Balto.; Consultant in Psychiatry, U. S. Dist. Court, Md. District, *2349 Eutaw Place, Baltimore 17, Md.*

RUDNICK, DOROTHEA, Ph.B., Ph.D., Assistant Professor of Biology, *Albertus Magnus College, 700 Prospect St., New Haven, Conn.*

RUGH, ROBERTS, M.A., Ph.D., Associate Professor of Radiology, Department of Radiology, *Columbia University, College of Physicians and Surgeons, 630 West 168th St., New York 32, N. Y.*

RUNNER, MEREDITH N., Research Associate, *Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.*

Ruth, Edward S., M.D., F.A.C.S., *1680 North Vine St., Hollywood, Calif.*

Ruth, Elbert B., A.B., A.M., Ph.D., Associate Professor of Anatomy, *University of North Dakota, Grand Forks, North Dakota.*

Ryerson, Dwight L., B.A., M.S., Ph.D., Assistant Professor of Zoology, *Pomona College, Claremont, Calif.*

Sabin, Florence R., B.S., M.D., Sc.D., LL.D. (Second Vice-Pres. '08–'09, Pres. '24–'26), Member Emeritus of Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City, *1333 East 10th Ave., Denver, Colo.*

Saccomanno, Geno, Ph.D., M.D., Pathologist and Director Clinical Laboratories, Veterans and St. Mary's Hospitals, *Grand Junction, Colo.*

Salmon, Theodora N., B.A., M.A., Ph.D., Instructor, Department of Biological Sciences, Hunter College; Research Associate, Columbia University, *19 Essex Ave., Montclair, N. J.*

Salsbury, C. R., M.D., C.M., F.R.C.S.(C.), F.R.C.S.(Eng.), *4784 W. 7th Ave., Vancouver, B. C., Canada.*

Sámano Bishop, Amelia, A.B., M.A., Ph.D., Professor of Embryology, Medical School; Professor Histology and Embryology, Graduate School, National University of Mexico. Research Histologist of the Instititute of Biology, National University of Mexico. *Institute of Biology, Casa del Lago, Chapultepec, Mexico D. F., Mexico.*

Sandstrom, Carl J., Ph.D., Associate Professor of Biology, *New York University, New York City 53.*

Sanford, Katherine Koontz, B.A., M.A., Ph.D., Cytologist, *National Cancer Institute, Bethesda, Md.*

Sano, Machteld Elisabeth, C.Sc., M.D., Associate Professor of Research Pathology, *Women's Medical College and Hospital of Pennsylvania, 515 West Chelten Ave, Philadelphia 44, Penna.*

Sattler, Dwight G., B.S., M.D., *Kalona, Iowa.*

Sauer, Mary E., A.B., A.M., Ph.D., Assistant Professor of Anatomy, University of Texas, *1115 Ave. E., Galveston, Tex.*

Saunders, John B. deC. M., M.B., Ch.B., F.R.C.S., Professor of Anatomy, Chairman of Division of Anatomy, *University of California, San Francisco, Calif.*

Saunders, Richard L. deC. H., M.B., Ch.B., M.D., F.R.S.E., F.R.S.A., Professor and Head of the Department of Anatomy, *Dalhousie University, Halifax, Nova Scotia, Canada.*

Sawin, Paul B., B.S., M.Sc., Sc.D., Research Associate, *R. B. Jackson Memorial Laboratory, Hamilton Station. Box 847, Bar Harbor, Maine.*

Sawyer, Charles H., A.B., Ph.D., Professor of Anatomy, *Duke University School of Medicine, Durham, N. C.*

Scammon, Richard E., Ph.D., LL.D. (Ex. Com. '23–'26, '35–'39, Vice-Pres. '28–'30), Distinguished Service Professor in the Graduate School, *172 S. E. Bedford St., Minneapolis, Minn.*

Schadewald, Melvin, B.S., M.S., Ph.D., Laboratory Director, Neurology, Mental Health Program, *825 — 5th St., S.E. Minneapolis 14, Minn.*

Schaeffer, Bobb, A.B., A.M., Ph.D., Assistant Curator of Fossil Vertebrates, *The American Museum of Natural History, Central Park West at 79th St., New York 24, N. Y.*

SCHAEFFER, JACOB PARSONS, A.M., M.D., Ph.D., Sc.D. (Ex. Com. '24–'29, Vice-Pres. '42–'43; Acting Pres. '43–'46), Professor of Anatomy and Director of the Daniel Baugh Institute of Anatomy, Emeritus Jefferson Medical College, *1025 Walnut St., Philadelphia 7, Pa.*

SCHARRER, ERNST, M.D., Ph.D., Associate Professor of Anatomy, *University of Colorado, School of Medicine, 4200 East 9th Ave., Denver 7, Colorado.*

SCHLEGEL, JØRGEN ULRIK, M.D., Ph.D., Assistant Professor of Urologic Physiology and Director of Urological Research Laboratory, University of Rochester, *Strong Memorial Hospital, 260 Crittenden Blvd., Rochester 20, N. Y.*

SCHMIDT, IDA GENTHER, B.A., M.A., Ph.D., Associate Professor of Anatomy, *University of Cincinnati, College of Medicine, Eden Ave., Cincinnati, O.*

SCHOEMAKER, DANIEL M., B.S., M.D., Professor Emeritus of Anatomy and Director Emeritus of the Department, School of Medicine, St. Louis University, *1402 S. Grand Ave., St. Louis, Mo.*

SCHOTTÉ, OSCAR E., Lic. Sc. Phys. et Nat., Doct. Sc. Nat., Geneva, Switzerland, Professor of Biology, *Amherst College, Amherst, Mass.*

SCHOUR, ISAAC, B.S., D.D.S., M.S., Ph.D., Professor of Histology and Head of the Department, University of Illinois College of Dentistry, *808 South Wood St., Chicago, Ill.*

SCHULTZ, ADOLPH H., Ph.D., Associate Professor of Physical Anthropology, Department of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

SCHWARTZ, HENRY G., M.D., Professor of Neurological Surgery, Washington University School of Medicine, *Barnes Hospital, 600 S. Kingshighway, St. Louis 10, Mo.*

SCHWEIZER, MALVINA, Ph.D., Assistant Professor of Biology, Washington Square College, New York University, *Washington Square, New York City 3, N. Y.*

SCOTT, DONALD, JR., B.S., Ph.D., Assistant Professor of Anatomy, *University of Pennsylvania, School of Medicine, Philadelphia 4, Pennsylvania.*

SCOTT, GORDON H., A.B., Ph.D., (First Vice Pres. '50–), (Executive Committee '49–'50), Dean, Wayne University College of Medicine, Professor of Anatomy and Head of Department, *Wayne University College of Medicine, 1512 St. Antoine, Detroit 26, Mich.*

SCOTT, JOHN W., A.M., Ph.D., Professor of Zoology and Research Parasitologist, University of Wyoming, *1409 Garfield St., Laramie, Wyo.*

SCOW, ROBERT OLIVER, A.B., M.A., M.D., Senior Assistant Surgeon, *U. S. Public Health Service, National Institute of Health, Bethesda 14, Maryland.*

SEDWICK, H. JOBE, D.D.S., *Elderton, Pa.*

SEIB, GEORGE A., A.B., M.D., Assistant Professor of Anatomy, Washington University, *2323 Lafayette Ave., St. Louis, Mo.*

SELLING, LAURENCE, A.B., M.D., Professor of Medicine, University of Oregon Medical School, *6005 S. W. Hamilton St., Portland 5, Ore.*

SENSENIG, E. CARL,, B.A., M.S., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, Medical College of Alabama, Birmingham 5, Alabama.*

SETTLAGE, PAUL H., Ph.B., M.A., Ph.D., M.D., Associate Professor of Anatomy, *Department of Anatomy, University of Wisconsin, Science Hall, Madison 6, Wis.*

Severinghaus, Aura E., B.S., M.A., Ph.D., Associate Dean and Associate Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York 32, N. Y.*

Shafiroff, Benjamin G. P., M.D., Associate Professor of Anatomy, New York University, Bellevue Medical Center, *2202 Quentin Road, Brooklyn, N. Y.*

Shaner, Ralph Faust, Ph.D., F.R.S.C., Professor of Anatomy, *University of Alberta, Edmonton, Alberta, Canada.*

Shanklin, William M., Ph.D., Professor of Histology and Neuroanatomy, *American University of Beirut Medical School, Beirut, Lebanon.*

Shapiro, Harry H., D.M.D., Assistant Professor of Anatomy, *Columbia University, 630 West 168th St., New York City.*

Sharp, Clayton, M.D., Retired, *414 Powells Lane, Westbury, N. Y.*

Sheehan, Donal, M.D., D.Sc., M.R.S.C., School of Medicine, New York University, *477 First Ave., New York, N. Y.*

Sheinin, John J., B.S., M.S., Ph.D., M.B., M.D., Sc.D., President, and Chairman Department of Anatomy, *Chicago Medical School, 710 South Wolcott Ave., Chicago, Ill.*

Shields, Randolph T., A.B., M.D., *606 West Cork, Winchester, Virginia.*

Shryock, E. Harold, B.S., M.A., M.D., Associate Professor of Anatomy, Dean of Loma Linda Division, *College of Medical Evangelists, 115 N. San Bernardino St., Loma Linda, Calif.*

Shumway, Waldo, A.B., A.M., Ph.D., Dean, *Stevens Institute of Technology, Hoboken, New Jersey.*

Sicher, Harry, M.D., Professor of Anatomy and Histology, *Loyola University School of Dentistry, 1757 W. Harrison St., Chicago 12, Ill.*

Silver, Maurice L., Ph.D., M.D., *224 Thayer Street, Providence, Rhode Island.*

Simer, Parke H., B.Ed., M.A., Ph.D., Professor of Anatomy, *University of Illinois, 1853 W. Polk St., Chicago, Ill.*

Simkins, Cleveland S., A.B., Ph.D., Professor of Anatomy, Head of Department, Chicago College of Osteopathy, *5250 S. Ellis Ave., Chicago 15, Ill.*

Simpson, Miriam Elizabeth, A.B., M.A., Ph.D., M.D., (Exec. Com. '50–), Professor of Anatomy, Division of Anatomy and Institute of Experimental Biology, *University of California, 1133 Euclid Ave., Berkeley 4, Calif.*

Simpson, William L., B.S., Ph.D., M.D., Professor of Experimental Oncology, Wayne University, College of Medicine, Scientific Director, *Detroit Institute of Cancer Research, 4811 John R Street, Detroit 1, Michigan.*

Sinclair, John G., B.S., M.S., Ph.D., Professor of Histology and Embryology, *University of Texas, Medical Branch, Galveston, Tex.*

Singer, Edward, M.D., Associate Director, St. Clare's Hospital, *111 East 61st St., New York City.*

Singer, Marcus, B.S., A.M., Ph.D., Associate Professor of Anatomy, *Cornell University, Ithaca, New York.*

Smelser, George K., A.B., Ph.D., Associate Professor of Anatomy, *Columbia University College of Physicians and Surgeons, 630 West 168th St., New York 32, N. Y.*

Smith, Carlton G., B.A., M.Sc., M.D., Ph.D., Professor of Anatomy, *University of Toronto, Toronto 5, Canada.*

Smith, Christianna, A.M., Ph.D., Professor of Zoölogy, *Mount Holyoke College, South Hadley, Mass.*

Smith, David T., A.B., M.D., Associate Professor of Medicine and Professor of Bacteriology, Duke University, *Duke Hospital, Durham, N. C.*

Smith, Fern Wenonah, A.B., M.S., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Tennessee, School of Medicine, Memphis 3, Tenn.*

Smith, George Milton, A.B., M.D., Research Associate in Anatomy, Yale University School of Medicine, *Pine Orchard, Conn.*

Smith, Philip Edward, M.S., Ph.D., D.Sc. (Ex. Com. '34–'38), (Pres. '40–'42), Professor of Anatomy, College of Physicians and Surgeons, Columbia University, *632 West 168th St., New York City.*

Smith, R. Dale, B.S., M.S., Ph.D., Professor and Head of Department of Anatomy, *Creighton University, School of Medicine, Omaha 2, Neb.*

Smith, Wilbur Cleveland, B.A., M.D., Sc.D., *"Pine Hills," Pass Christian, Mississippi.*

Smith, Wilbur K., A.B., M.D., Associate Professor of Anatomy, *University of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Snider, Ray S., Ph.D., Associate Professor of Anatomy, *Northwestern University Medical School, Chicago, Ill.*

Snodgrass, L. E., A.B., M.D., Instructor in Surgery, Medical Department, University of Pennsylvania, *709 Walnut St., Philadelphia 6, Pa.*

Snook, Theodore, B.S., M.S., Ph.D., Assistant Professor of Anatomy, *University of Pittsburgh, School of Medicine, Pittsburgh 13, Pa.*

Snyder, Franklin Faust, M.D., Associate Professor of Anatomy and Obstetrics, *Harvard Medical School, 25 Shattuck St., Boston, Mass.*

Solnitzky, Othmar, A.B., A.M., Ph.D., M.D., Professor and Director of the Department of Anatomy, Georgetown University, School of Medicine; Director, Georgetown University Brain Research Institute, *3900 Reservoir Rd., Washington, D. C.*

Sosa, Julio Maria, M.D., Head, Division for Histological Research, *Neurological Institute, School of Medicine, University of Montevideo, Juan D. Jackson 1015, Montevideo, Uruguay.*

Spector, Benjamin, M.D., Professor of Anatomy, *Tufts College Medical School, 136 Harrison Ave., Boston 11, Mass.*

Speidel, Carl Caskey, Ph.B., Ph.D., Sc.D., (Ex. Com. '40–'46), Professor and Chairman of Anatomy, *Medical Department, University of Virginia, Charlottesville, Va.*

Sperry, Roger Wolcott, A.B., A.M., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Chicago, Chicago 37, Ill.*

Spofford, Walter R., B.S., Ph.D., Associate Professor of Neuroanatomy, *State University of New York Medical Center at Syracuse University, Syracuse 10, New York.*

Sprague, James Mather, A.B., A.M., Ph.D., Assistant Professor of Anatomy, *School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*

Spratt, Nelson T., Jr., A.B., Ph.D., Professor of Zoology, *Department of Zoology, University of Minnesota, Minneapolis 14, Minn.*

Stark, Mary B., Ph.B., M.A., Ph.D., Sc.D., *Harris, Minnesota.*

STEIN, KATHRYN F., M.S., Ph.D., Assistant Professor of Zoölogy, *Mount Holyoke College, South Hadley, Mass.*

STEINBERG, MINNIE HELLER, B.S., Ph.D., Research Assistant, *University of Chicago, 5526 S. Everett, Chicago 37, Illinois.*

STILWELL, E. FRANCES, A.B., A.M., Ph.D., Associate Professor of Anatomy, *Woman's Medical College of Pennsylvania, Philadelphia, Pa.*

STONE, LEON STANSFIELD, Ph.B., Ph.D., Bronson Professor of Comparative Anatomy, *Yale University School of Medicine, 333 Cedar St., New Haven, Conn.*

STOPFORD, JOHN SEBASTIAN B., M.D., Sc.D., D.Sc., F.R.S., Vice-Chancellor and Professor of Experimental Neurology, *University of Manchester, Manchester, England.*

STOTLER, WILLIAM A., A.B., M.S., Ph.D., Assistant Professor of Anatomy, *University of Oregon Medical School, Portland, Oregon.*

STOWELL, ROBERT E., A.B., M.D., Ph.D., Professor of Pathology and Oncology, *University of Kansas Medical Center, Kansas City 3, Kans.*

STRAUS, WILLIAM L., JR., A.B., Ph.D., Associate Professor of Anatomy, *Johns Hopkins Medical School, Baltimore 5, Md.*

STRAUSS, FRITZ, M.D., Privat Docent and Prosector, Department of Anatomy, *University of Berne, Buehlstrasse 26, Berne, Switzerland.*

STROMSTEN, FRANK ALBERT, B.S., M.S., D.Sc., Associate Professor Emeritus, Zoölogy, State University of Iowa, *318 Zoology Building, Iowa City, Ia.*

STRONG, LEON HENRI, A.B., B.S., Ph.D., Associate Professor of Anatomy, *Chicago Medical School, 710 S. Wolcott Ave., Chicago 12, Ill.*

STRONG, REUBEN MYRON, A.B., A.M., Ph.D., (Ex. Com. '16–'19), Chairman and Professor Emeritus, Loyola University School of Medicine; Research Associate, *Chicago Natural History Museum, Roosevelt Rd. and Lake Shore Dr., Chicago 5, Ill.*

STULTZ, WALTER A., B.A., Ph.D., Associate Professor of Anatomy, *University of Vermont, College of Medicine, Burlington, Vt.*

STUNKARD, H. W., B.S., A.M., Ph.D., Sc.D., Professor of Biology, *New York University, University Heights, New York City.*

SULKIN, NORMAN M., M.A., Ph.D., Assistant Professor of Anatomy, *St. Louis University Medical School, 1402 South Grand Boulevard, St. Louis, Mo.*

SULLIVAN, WALTER EDWARD, A.M., Ph.D., (Ex. Com. '41–'48), Professor of Anatomy, University of Wisconsin, *Science Hall, Madison, Wis.*

SUNDBERG, R. DOROTHY, B.S., M.A., Ph.D., Assistant Professor of Anatomy; Staff Hematologist, University of Minnesota Hospitals; *319 Institute of Anatomy, University of Minnesota, Minneapolis 14, Minn.*

SUNDERLAND, SYDNEY, B.S., M.B., D.Sc., M.D., Professor of Anatomy and Histology, *University of Melbourne, Carlton N. 3, Melbourne, Vic., Australia.*

SWANK, ROY LAVER, Ph.D., M.D., Assistant Professor of Experimental Neurology, *McGill University, 3801 University St., Montreal, Canada.*

SWIFT, CHARLES H., M.D., Ph.D., Associate Professor of Anatomy, Emeritus, *Department of Anatomy, The University of Chicago, Chicago, Ill.*

SWINGLE, W. W., A.B., A.M., Ph.D., Professor of Biology, *Princeton University, Princeton, N. J.*

SWINYARD, C. A., B.S., M.S., Ph.D., Professor of Anatomy, *College of Medicine, University of Utah, Salt Lake City, Utah.*

SYKES, GEORGE F., Ph.B., M.A., Assistant Professor of Anatomy, Tufts College Medical School, *136 Harrison Ave., Boston 11, Mass.*

SZEPSENWOL, JOSEL, M.D., Associate Professor, Department of Anatomy, *Emory University School of Medicine, Emory University, Ga.*

TALLMADGE, G. KASTEN, A.M., M.D., Ph.D., Assistant Professor of Anatomy, Marquette University School of Medicine, *561 North Fifteenth Street, Milwaukee 3, Wisconsin.*

TAYLOR, A. CECIL, B.A., M.A., Ph.D., Associate Professor of Anatomy, College of Dentistry and Graduate School of Arts and Science, *New York University, 477 First Avenue, New York 16, N. Y.*

TEITELBAUM, HARRY A., B.S., M.D., Ph.D., Assistant in Neurology, The Johns Hopkins University Medical School; Associate in Neurology, University of Maryland School of Medicine, *1801 Eutaw Pl., Baltimore 17, Md.*

TELFORD, IRA ROCKWOOD, A.B., M.A., Ph.D., Chairman and Professor in Anatomy, Department of Anatomy, School of Dentistry, University of Texas, *1018 Blodgett St., Houston 4, Texas.*

TERNI, TULLIO, M.D., Professor of Anatomy, *The University of Padua, Padua, Italy.*

TERRILL, CLAIR ELMAN, B.S., Ph.D., Animal Husbandman (Physiologist and Geneticist), *U. S. Western Sheep Breeding Laboratory, Dubois, Idaho.*

TERRY, ROBERT JAMES, A.B., M.D., (Ex. Com. '08–'12, '21–'24), Professor of Anatomy, Emeritus, *Washington University School of Medicine, St. Louis, Mo.*

THOMAS, THURLO B., S.B., M.A., Ph.D., Professor of Zoology and Chairman, *Department of Zoological Sciences, Carleton College, Northfield, Minn.*

THOMPSON, I. MACLAREN, B.Sc., M.B., Ch.B., F.R.S.C., Professor of Anatomy and Director of the Department of Anatomy, *Medical College, University of Manitoba, Winnipeg, Canada.*

THOMSON, STEWART CRAIG, A.B., M.S., M.D., M.P.H., Associate Professor and Assistant Director, *School of Public Health, University of Minnesota, 121 Millard Hall, Minneapolis, Minn.*

THOREK, PHILIP, M.D., B.S., F.A.C.S., F.I.C.S., Assistant Professor of Surgery, University of Illinois, Associate Professor of Surgery, Cook County Graduate School, *25 E. Washington Street, Chicago 2, Illinois.*

THURINGER, JOSEPH M., M.D., Professor of Histology and Embryology, University of Oklahoma, School of Medicine, *801 N. E. 13th St., Oklahoma City, Okla.*

TOBIN, CHARLES E., Ph.D., Associate Professor of Anatomy, *The University of Rochester, School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N. Y.*

TOMPKINS, EDNA H., M.D., Research Associate, *Cancer Research Institute, New England Deaconess Hospital, 195 Pilgrim Road, Boston 15, Mass.*

TOWER, SARAH SHELDON, B.S., Ph.D., M.D., Instructor in Psychiatry, Johns Hopkins University, *11 E. Chase St., Baltimore 2, Md.*

TRACY, HENRY C., A.B., A.M., Ph.D., Professor of Anatomy, *University of Kansas, Lawrence, Kan.*

TRENTIN, JOHN J., B.S., A.M., Ph.D., Jane Coffin Childs Fellow, *Department of Anatomy, Yale Medical School, New Haven, Conn.*

TROTTER, MILDRED, A.B., M.S., Ph.D., Professor of Gross Anatomy, *Washington University School of Medicine, St. Louis, Mo.*

TRUEX, RAYMOND C., M.S., Ph.D., Professor and Chairman, Division of Anatomy, *The Hahnemann Medical College and Hospital of Philadelphia, 235 N. 15th St., Philadelphia 2, Pa.*

TRUSCOTT, BASIL LIONEL, B.A., M.A., M.S., Ph.D., *Duke Hospital, Durham, N. C.*

TSANG, YU-CHUAN, B.A., Ph.D., Professor of Anatomy, *The Medical College, Peking National University, Peking, China.*

TSOURAS, SOTIRIOS JOHN, M.D., Dr. of Anatomy, Instructor, *Department of Anatomy, University of Michigan, Ann Arbor, Mich.*

TUNTURI, ARCHIE ROBERT, A.B., M.S., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Oregon Medical School, Portland, Oregon.*

TURNER, CHARLES W. B.S., A.M., Ph.D., Professor of Dairy Husbandry, *203 Eckles Hall, University of Missouri, Columbia, Mo.*

TURNER, C. DONNELL, Ph.D., Chairman, Department of Zoology, *Utica College of Syracuse University, Utica, New York.*

TURNER, CLARENCE L., M.A., Ph.D., Professor of Zoology, Cresap Laboratory, *Northwestern University, Evanston, Ill.*

TURNER, ROBERT STUART, A.B., Ph.D., Associate Professor of Anatomy, *Stanford University, Calif.*

UHLENHUTH, EDUARD, Ph.D., Professor of Anatomy, *University of Maryland Medical School, Baltimore, Md.*

UOTILA, UNTO U., M.D., Research Fellow in Anatomy, Harvard Medical School, Boston, Mass. (1939); Docent in Microscopical Anatomy, *Helsinki University, Siltavuorenpenger 20, Helsinki, Finland.*

VAN BUSKIRK, CHARLES, Ph.D., M.D., Resident in Neurology, University of Minnesota, *1920 Stevens Ave. So., Minneapolis, Minn.*

VAN CAMPENHOUT, ERNEST, M.D., Ph.D., Professor of Anatomy, Histology and Embryology, *Université de Louvain, Louvain, Belgium.*

VAN CLEAVE, C. D., Ph.D., Associate Professor of Anatomy, *School of Medicine, University of North Carolina, Chapel Hill, N. C.*

VAN DER HORST, C. J., D.Sc., Professor, Head of the Department of Zoölogy, *University of the Witwatersrand, Johannesburg, So. Africa.*

VAN DYKE, JOHN H., A.B., M.S., Ph.D., Associate Professor of Anatomy, *Indiana University, School of Medicine, Bloomington, Ind.*

VAN WAGENEN, GERTRUDE, A.B., Ph.D., Research Associate in Obstetrics and Gynecology, Associate Professor's rank, Yale University, *New Haven Hospital, New Haven, Conn.*

VICARI, EMELIA, A.B., A.M., Staff Investigator, *Roscoe B. Jackson Laboratory, Bar Harbor, Me.*

VON BONIN, GERHARDT, M.D., Professor of Anatomy, University of Illinois, College of Medicine, *1853 West Polk St., Chicago, Ill.*

VONDERAHE, A. R., A.B., B.Sc., M.D., Associate Professor of Anatomy, *University of Cincinnati, College of Medicine, Eden Ave., Cincinnati, O.*

WAITE, FREDERIK CLAYTON, A.M., Ph.D., Emeritus Professor of Histology and Embryology, Western Reserve University School of Medicine, *144 Locust St., Dover, N. H.*

WALDO, CHARLES MERREL, B.S., D.D.S., M.S., Associate Professor of Orthodontics, *Harvard School of Dental Medicine, 25 Shattuck St., Boston 15, Mass.*

WALLER, HENRY, Ph.D., M.D., Associate Professor of Anatomy, Boston University Medical School, *80 E. Concord St., Boston 18, Mass.*

WALLIN, IVAN E., M.A., D.Sc., Professor of Anatomy and Head of Department, *University of Colorado, School of Medicine, Denver, Colo.*

WALLS, G. L., B.S., A.M., Sc.D., Associate Professor Physiological Optics and Optometry, *School of Optometry, University of California, Berkeley 4, Calif.*

WALMSLEY, THOMAS, M.D., F.R.S.E., Professor of Anatomy, *Queen's University of Belfast, Belfast, Ireland.*

WARBRITTON, VIRGENE, Ph.D., (Mrs. Fred Kavanagh), *New York Botanical Gardens, New York 58, N. Y.*

WARD, JAMES W., Ph.D., M.D., Associate Professor of Anatomy, *Vanderbilt University Medical School, Nashville, Tenn.*

WARD, JAMES WELLINGTON, A.B., M.A., M.S., M.S., Ph.D., Associate Professor, *Department of Anatomy, School of Medicine, University of Mississippi, Box 183, University, Mississippi.*

WARNER, FRANCIS JAMES, B.A., M.A., M.D., Fellow in Neurology at the Henry Ford Hospital, *P.O. Box 135, North End Station, Detroit 2, Michigan.*

WASHBURN, SHERWOOD L., A.B., Ph.D., Associate Professor of Physical Anthropology, *University of Chicago, Chicago 37, Ill.*

WASSERMANN, FRIEDRICH, M.D., Professor of Anatomy Emeritus, The University of Chicago; Senior Biologist, Biology Division, Argonne National Laboratory, *P.O. Box 5207, Chicago 80, Illinois.*

WATERMAN, ALLYN J., A.B., A.M., Ph.D., Professor of Biology, *Williams College, Williamstown, Mass.*

WATKINS, RICHARD WATKIN, S.B., M.D., Assistant Professor of Otolaryngology, Rush Medical College, *5608 Kenwood Ave., Chicago 37, Ill.*

WATT, JAMES CRAWFORD, M.A., M.D., Professor of Anatomy, *University of Toronto; 105 Whitehall Rd., Toronto 5, Ontario, Canada.*

WATTERSON, RAY L., B.A., Ph.D., Associate Professor of Biology, *Department of Biology, Northwestern University, Evanston, Ill.*

WEAVER, HARRY M., A.B., M.Sc., Ph.D., Director of Research, The National Foundation for Infantile Paralysis, *120 Broadway, New York 5, N. Y.*

WEBB, RICHARD L., A.B., M.S., Ph.D., Professor of Anatomy and Chairman, *Indiana University, 209 Med. Bldg., Bloomington, Ind.*

WEBER, NEAL A., B.A., M.S., M.A., Ph.D., Associate Professor of Zoology, *Swarthmore College, Swarthmore, Pa.*

WEED, LEWIS HILL, A.B., A.M., M.D., Sc.D., LL.D., (Ex. Com. '20–'21, '35–'39, Sec.-Treas. '22–'30), *720 Centre Ave., Reading, Pa.*

WEICHERT, CHARLES KIPP, B.S., M.S., Ph.D., Professor of Zoology, *University of Cincinnati, Cincinnati 21, O.*

WEINSTEIN, B. BERNARD, B.S., M.D., M.S., Associate Professor of Obstetrics and Gynecology, Tulane University, *1441 Delachaise St., New Orleans 15, La.*

WEISS, PAUL ALFRED, Ph.D., M.D. (h.c.), Professor of Zoology, *The University of Chicago, Chicago, Ill.*

WEISSENBERG, RICHARD, M.D., Visiting Professor of Anatomy, Department of Anatomy, *Women's Medical College of Pennsylvania, Philadelphia 29, Pa.*

WELLS, LEMEN J., A.M., Ph.D., Professor of Anatomy, *Institute of Anatomy, University of Minnesota, Minneapolis 14, Minn.*

WESTON, JEAN K., A.B., M.S., Ph.D., M.D., Research Pathologist, *Research Department, Parke, Davis and Co., Detroit 32, Mich.*

WHEATLEY, MAX D., A.B., M.A., Ph.D., Assistant Professor Department of Anatomy, *College of Medicine, State University of Iowa, Iowa City, Iowa,*

WHEELER, THEODORA, A.B., M.D., *The Knoll-Lake Shore Dr., Lake Geneva, Wis.*

WHITAKER, WAYNE L., A.B., A.M., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, University of Michigan, Ann Arbor, Mich.*

WHITE, ELIZABETH LLOYD, A.B., M.A., Ph.D., Instructor, *Wheaton College, Norton, Mass.*

WHITEHEAD, (MRS.) PEARL CLAUS, B.A., M.A., Ph.D., Assistant Professor of Zoology, *Biology Building, University of Wisconsin, Madison 6, Wis.*

WHITESIDE-HAWEL, BEATRICE, B.A., Ph.D., *c/o Guaranty Trust Co., 140 Broadway, New York City.*

WHITTIER, JOHN RENSSELAER, B.A., M.D., Research Assistant, *Department of Neurology, Columbia University, 630 West 168th Street, New York City 32, N. Y.*

WIEMAN, H. L., Ph.D., Professor of Zoology, Emeritus, University of Cincinnati, *Falmouth, Mass.*

WIERDA, JAY L., Ph.D., Assistant Professor of Anatomy, *School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*

WILCOX, HARRY H., B.S., M.S., Ph.D., Associate in Anatomy, *University of Pennsylvania, School of Medicine, Philadelphia 4, Pa.*

WILLARD, WILLIAM A., A.M., Ph.D., Professor of Anatomy, University of Nebraska, *College of Medicine, Forty-second St. and Dewey Ave., Omaha, Neb.*

WILLIAMS, GEORGE D., A.B., A.M., M.D., Ph.D., *3495 Bailey Ave., Buffalo 15, N. Y.*

WILLIAMS, ROY GRIFFIN, A.M., M.D., Professor of Anatomy, *University of Pennsylvania School of Medicine, Philadelphia 4, Pa.*

WILLIAMS, STEPHEN CULVER, Ph.D., Assistant Professor of Anatomy, *Medical Department, University of Pennsylvania, Philadelphia 4, Pa.*

WILLIAMS, T. WALLEY, JR., Ph.D., Associate Professor of Anatomy and Histology, *School of Medicine, West Virginia University, Morgantown, W. Va.*

WILLIAMS, W. LANE, B.S., M.A., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, University of Minnesota, Minneapolis 14, Minnesota.*

WILLIER, BENJAMIN HARRISON, Ph.D., D.Sc., Henry Walters Professor of Zoology and Chairman of Department of Biology, *Johns Hopkins University, Charles and 34th Sts., Baltimore 18, Md.*

WILSON, JAMES G., B.A., M.A., Ph.D., Associate Professor of Anatomy, *University of Cincinnati College of Medicine, Cincinnati, Ohio.*

WILSON, J. WALTER, Ph.B., Ph.D., Day Professor of Biology and Chairman of Biology Department, *Brown University, Providence 12, Rhode Island.*

WIMSATT, WILLIAM ABELL, A.B., Ph.D., Associate Professor of Zoology, *Department of Zoology, Cornell University, Ithaca, N. Y.*

WINDLE, WILLIAM F., M.S., Ph.D., Sc.D., Scientific Director, *Baxter Laboratories, Inc., Morton Grove, Ill.*

WISLOCKI, GEORGE B., A.B., M.D., A.M., (hon.), (Ex. Com. '37–'41, First Vice-Pres. '48–'50), James Stillman Professor of Comparative Anatomy and Hersey Professor of Anatomy, *Harvard Medical School, Boston, Mass.*

WITSCHI, EMIL, Ph.D., Professor of Zoology, *State University of Iowa, Iowa City, Ia.*

WOERNER, CHARLES A., Ph.D., M.D., Associate Professor of Anatomy, *School of Medicine, University of Louisville, Louisville 2, Ky.*

WOLFE, HAROLD RECLUS, B.S., M.S., Ph.D., Professor and Chairman, Department of Zoology, *Biology Building, University of Wisconsin, Madison 6, Wis.*

WOLFE, J. M., B.S., Ph.D., Professor of Anatomy, and Director of the Department, *Albany Medical College, Albany 3, N. Y.*

WOLFF, DOROTHY, A.B., M.A., Ph.D., Research Histopathologist, *Lempert Institute of Otology, 119 E. 74th Street, New York City, N. Y.*

WOO, JU-KANG, M.S., Ph.D., Teaching Human Anatomy, Dairen University School of Medicine, Dairen, China, *c/o Dept. of Anatomy, Washington University School of Medicine, St. Louis 10, Mo.*

WOODBURNE, RUSSELL T., M.A., Ph.D., Professor of Anatomy, *University of Michigan, 1002 E. Medical Bldg., Ann Arbor, Mich.*

WOOLLEY, GEORGE WALTER, B.S., M.S., Ph.D., Head of Division of Steroid Biology, *Sloan-Kettering Institute, Memorial Hospital Center, 444 East 68th St., New York 21, New York.*

WORLEY, LEONARD GEORGE, A.B., M.A., Ph.D., Associate Professor of Biology, Chief Pre-medical Counselor, *Department of Biology, Brooklyn College, Brooklyn 10, New York.*

WRIGHT, PHILIP LINCOLN, B.S., M.S., Ph.D., Associate Professor of Zoology, *Montana State University, Missoula, Montana.*

WRIGHT, WALTER H., D.D.S., B.S., M.S., Ph.D., Dean, New York University College of Dentistry, *209 E. 23rd St., New York 10, N. Y.*

WYBURN, GEORGE M., D.Sc., M.B., F.R.F.P.S.G., F.R.S.E., Regius Professor of Anatomy, *Anatomy Department, The University, Glasgow, W. 2., Scotland.*

YEAKEL, ELEANOR H., A.B., M.A., Ph.D., Assistant Professor, *Department of Anatomy, Hahnemann Medical College, 235 No. 15th St., Philadelphia 2, Pa.*

YNTEMA, C. L., Ph.D., Professor of Anatomy, *School of Medicine, State University of New York, Syracuse 10, N. Y.*

YOFFEY, J. M., D.Sc., M.D., F.R.C.S., Professor of Anatomy, *The University, Bristol, Great Britain.*

YOSS, ROBERT EUGENE, M.D., Instructor in Anatomy, *University of Michigan Medical School, Ann Arbor, Michigan.*

YOUNG, M. WHARTON, B.S., M.D., Ph.D., Professor of Anatomy, College of Medicine, *Howard University, Washington, D. C.*

YOUNG, WILLIAM C., Ph.D., Professor of Anatomy, *University of Kansas, Lawrence, Kansas.*

YOUNGSTROM, KARL A., A.B., M.A., Ph.D., M.D., Major M.C., *Letterman General Hospital, Presidio, San Francisco, Calif.*

ZACHARIAS, LEONA RUTH, B.A., M.A., Ph.D., Instructor in Anatomy, *32 Clifton St., Belmont 78, Mass.*

ZECHEL, GUSTAV, M.D., F.A.C.S., Assistant Professor in Anatomy and Surgery, University of Illinois College of Medicine, *1853 W. Polk St., Chicago, Ill.*

ZEIT, WALTER, B.S., M.S., Ph.D., Professor of Anatomy, *Marquette University School of Medicine, 561 North 15th St., Milwaukee 3, Wis.*

ZIMMERMANN, ARNOLD, Lic.Sc., D.Sc., (Geneva, Switzerland), Professor of Anatomy, University of Illinois College of Medicine, *1853 W. Polk St., Chicago, Ill.*

ZITZLSPERGER, SIGFRID, Aerztliches Staatsezamen, Dr. Med., Dr. Med. Habil., Instructor in Anatomy, *University of Tennessee, Memphis 3, Tennessee.*

ZORZOLI, ANITA, A.B., M.A., Ph.D., Assistant Professor of Biochemistry and Physiology, School of Dentistry, *Washington University, St. Louis 10, Mo.*

ZWEIFACH, BENJAMIN WILLIAM, B.S., M.S., Ph.D., Assistant Professor of Physiology, *Department of Medicine, Cornell University Medical College, 1300 York Ave., New York 21, N. Y.*

ZWEMER, RAYMUND L., A.B., Ph.D., Chief, *Science Division and Consultant in Biology, Library of Congress, Washington 25, D. C.*

1951

MOTION PICTURE FILMS AT THE WISTAR INSTITUTE

Approved by a Review Committee of the American Association of Anatomists or the American Society of Zoologists or The Wistar Institute. They are available to scientists and students for use at The Wistar Institute free of charge. Sale and rental terms for use elsewhere of 16 mm copies are given below. Unless otherwise noted, all films are monchrome, silent. Films distributed by The Wistar Institute are not rented outside continental United States.

SUBJECT AND TITLE	REELS OR LENGTH	PRODUCER	TERMS	DISTRIBUTOR
CYTOLOGY				
Dividing normal adult rat fibroblasts in tissue cultures	160 ft.	W. H. Lewis	Rental, $2.00 per day Sale, $10.00	The Wistar Institute 36th & Woodland Ave. Philadelphia 4, Pa.
Dividing cancer cells	160 ft.	ditto	Rental, $2.00 per day Sale, $10.00	ditto
Cultures of normal and cancer cells. Rat sarcomas and carcinomas	1	ditto	Rental, $4.00 per day Sale, $25.00	ditto
Pinocytosis. Drinking by cells	264 ft.	ditto	Rental, $3.00 per day Sale, $16.50	ditto
Growth of capillaries and endothelium	140 ft.	ditto	Rental, $2.00 per day Sale, $10.00	ditto
Normal and abnormal white blood cells in tissue cultures	1	W. H. Lewis M. R. Lewis A. R. Rich M. M. Wintrobe	Rental, $4.00 per day Sale, $25.00	ditto
Nerve fibers and neuroglia in tissue cultures	1	W. H. Lewis M. R. Lewis	ditto	ditto
Locomotion of Chaos chaos, the giant ameba	1	W. H. Lewis	ditto	ditto
Locomotion of the slime mold, Phasarum polycephalum	1	ditto	ditto	ditto
Activity of heart muscle, from rat embryos, grown in tissue culture	1	C. M. Goss		Dr. C. M. Goss 1542 Tulane Ave. New Orleans, La.
EMBRYOLOGY				
The early development of the rabbit egg	1	W. H. Lewis P. W. Gregory	ditto	The Wistar Institute 36th & Woodland Ave. Philadelphia 4, Pa.
The early development of the rabbit egg (short film — selected scenes from the long film)	80 ft.	ditto	Rental, $1.50 per day Sale, $5.00	ditto
Cleavage, 2–8 cells, of living monkey egg in vitro	80 ft.	W. H. Lewis C. G. Hartman	ditto	ditto

Price of reel and can with purchased film, $1.00

SUBJECT AND TITLE	REELS OR LENGTH	PRODUCER	TERMS	DISTRIBUTOR
Embryology (Cont.)				
Development of Zebra fish egg	1	W. H. Lewis E. C. Roosen-Runge	Rental, $4.00 per day Sale, $25.00	ditto
Gastrulation of Amblystoma punctatum	1	W. H. Lewis	ditto	ditto
Development of a salamander	1	L. S. Stone T. C. Kramer		Films, Inc. 330 W. 42nd St. New York, N. Y.
Where chick life begins (kodachrome, silent)	1	A. L. Romanoff		Ralston Purina Co. St. Louis, Mo.
GENERAL				
Unseen worlds (monochrome, sound)	1	RCA Victor Camden, N. J.		Institute of Visual Training 40 E. 49th St. New York 17, N. Y.
The eyes of science	3	Bausch & Lomb Optical Co.		
Heredity	1	H. Strandskov		Encyclopaedia Britannica Films, Inc. 20 N. Wacker Drive Chicago 6, Ill.
High speed motion pictures taken with stroboscopic light	1	H. E. Edgerton K. J. Germeshausen H. E. Grier		
Seeing the unseen	1	H. E. Edgerton		
The management of cicatricial strictures of the esophagus (kodachrome, silent)	1	E. H. Ingersoll		
Surgical anatomy (kodachrome, silent)	1	C. J. Baumgartner Med. Director		Billy Burke Productions 7416 Beverly Blvd. Hollywood, Calif.
Congenital anomalies	2	J. Sarnoff		Sarnoff Motion Picture Film Library 1406 Albemarle Rd. Brooklyn, N. Y.
PHYSIOLOGY				
Alimentary tract	1	A. J. Carlson H. G. Swann		Encyclopaedia Britannica Films, Inc. 20 N. Wacker Drive Chicago 6, Ill.
Digestion of foods	1	ditto		ditto
Endocrine glands	1	ditto		ditto
The heart and circulation	1	A. J. Carlson		ditto
Work of the kidneys	1	A. J. Carlson H. G. Swann F. J. Mullin		ditto
Mechanisms of breathing (monochrome, sound)	1	V. Johnson		ditto

SUBJECT AND TITLE	REELS OR LENGTH	PRODUCER	TERMS	DISTRIBUTOR
Physiology (Cont.)				
How the eye functions	1	John R. McCrory Studios		Knowledge Builders 625 Madison Ave. New York, N. Y.
How the ear functions	1	ditto		ditto
Our teeth	1	ditto		ditto
The nervous system (5 parts)	14	I. V. Pavlov and others		Brandon Films, Inc. 1700 Broadway New York, N. Y.
Effect of electrical stimulation of the right ansiform lobe of the cerebellum of the cat (Felis domestica)	100 ft.	S. L. Clark		Dr. Sam L. Clark Department of Anatomy Vanderbilt University Nashville, Tenn.
The control of small blood vessels	1	G. P. Fulton B. R. Lutz		Dr. G. P. Fulton College of Liberal Arts Boston University Boston, Mass.
Mesenteric lymphatics, their conduct and the behavior of their valves in the living rat	1	R. L. Webb	Rental, $4.00 per day	The Wistar Institute 36th & Woodland Ave. Philadelphia 4, Pa.
Effect of various drugs on contractions of mesenteric lymphatics in the rat	300 ft.	ditto	Rental, $3.00 per day	ditto
REPRODUCTION				
Spermatozoa — fish, frog, snake, rooster, turkey, rat and hamster (phase microscopy) 1951	1	E. J. Farris	Sale; rental $4.00 per day	ditto
Spermatozoa — opossum, rabbit, pig, cat, dog, bull, monkey (phase microscopy) 1951	1	ditto	Sale; rental $4.00 per day	ditto
Human spermatozoa: morphology and motility (phase microscopy) 1951 revision	1	ditto	Sale; rental $4.00 per day	ditto
Human semen analysis. Technique for counting motile sperm. 1951 revision	225 ft.	ditto	Sale; rental $2.25 per day	ditto
Human semen analysis. Motile spermatozoa as an index of fertility in man. 1951 revision	425 ft.	ditto	Sale; rental $4.25 per day	ditto
Development of the opossum (kodachrome, silent)	150 ft.	ditto	Rental, $3.00 per day	ditto
Tropical opossums (kodachrome, silent)	1	ditto	Rental, $8.00 per day	ditto
The biology of reproduction in rats (kodachrome, silent)	1	ditto	ditto	ditto

SUBJECT AND TITLE	REELS OR LENGTH	PRODUCER	TERMS	DISTRIBUTOR
Reproduction (Cont.)				
The timing of human ovulation (kodachrome, silent)	1 (450 ft.)	ditto	Sale Rental, $9.00 per day	The Wistar Institute 36th & Woodland Ave. Philadelphia 4, Pa.
Proestrous and oestrous behavior in the guinea pig	150 ft.	W. C. Young E. W. Dempsey R. Hertz and H. L. Myers	Rental, $2.00 per day Sale	ditto
Studies in human fertility (monochrome, sound)	1 (2000 ft.)	Audio Productions, Inc.		Ortho Pharmaceutical Corp., Raritan, N. J.
Reproduction among mammals	1	M. Brodshaug F. T. Howard H. H. Strandskov		Encyclopaedia Britannica Films, Inc. 20 N. Wacker Drive Chicago 6, Ill.
Maternal behavior in the rat and its modification by cortical injury	350 ft.	F. A. Beach		Yale University New Haven, Conn.
Mating behavior in the leopard frog, Rana pipiens	250 ft.	F. A. Beach E. R. Aronson		American Museum of Natural History New York, N. Y.
Ovulation in the frog, Rana pipiens	1	Dr. R. Rugh		Columbia University Dept. of Radiology 630 W. 168th St. New York 32, N. Y.

The Wistar Institute will publish from time to time a list of approved films given to The Wistar Institute for its Film Depository. Each film should be accompanied by a script. For sale and rental terms, consult distributor.

FILM REVIEWS

Films submitted for review to either the Committee for the American Association of Anatomists or the American Society of Zoologists, should be sent with script to Dr. E. J. Farris, The Wistar Institute. If the film is approved, a film review will appear in the Anatomical Record.

THE GLYCOGEN CONTENT OF VARIOUS TISSUES OF THE CHICK EMBRYO

WILLIAM H. LEE
Department of Anatomy, Emory University School of Medicine, Emory University, Georgia[1]

ONE FIGURE

INTRODUCTION

The question of whether the carbohydrate metabolism in the embryo is the same as in the adult is still controversial. According to Needham and Nowinski ('31, '36, and '37) the chick embryo has an active system for the utilization of glucose, but lacks some of the enzymes necessary for the metabolism of glycogen and hexosephosphate. On the contrary, Novikoff, Potter, and LePage ('48) think that the embryo, like the adult, is able to use glycogen and possesses all the enzymes involved in the breakdown of the latter. They demonstrated the presence of glycogen and of practically all the intermediates involved in its breakdown to lactic acid by chemical analysis of 3 to 10 day old chick embryos. Moreover, they found that homogenates of embryos 5 to 8 days old utilize hexosediphosphate, fructose-6-phosphate, and glucose.

In view of the above controversy and of the recent observation of Szepsenwol ('46) that embryonic muscle undergoes certain physiological changes between the 13th and 18th days of incubation, it was thought that a study of the glycogen distribution in the various tissues of the embryo throughout development would be of interest. Muscle of chick embryos one to 13 days old becomes spontaneously active when cultivated *in vitro,* while that from embryos older

---

[1] The author gratefully acknowledges his indebtedness to Dr. J. Szepsenwol for guidance throughout this investigation.

THE ANATOMICAL RECORD, VOL. 110, NO. 4
AUGUST 1951

than 18 days grows and, like adult muscle, responds to electrical stimuli, but shows no spontaneous activity. The spontaneous contractibility of embryonic muscle begins to diminish after the 13th day of incubation and the physiological characteristics of adult muscle gradually become evident. Consequently, it seems likely that this transformation might be accompanied by changes in the content of glycogen and phosphate, the substances of primary importance in muscle activity.

This paper describes the changes observed in the glycogen concentration of liver, of heart, and of skeletal muscle throughout development of the chick embryo.

HISTORICAL REVIEW

Many authors have studied the glycogen content of chick embryos at various stages of development, but all analyzed either the embryo in its entirety (Sakuragi, '17; Idzumi, '24; and Murray, '25) or made separate extracts of the liver and of the rest of the body (without liver) (Vladimirov and Danilina, '30; Gill, '38).

Donhoffer ('33) fractionated the carbohydrates of the embryo by alcohol extraction, and found that only 10 mg of glycogen are present in the egg at the beginning of incubation. This value increases to 90 mg at hatching.

Gill ('38) found that the liver glycogen increases gradually with embryonic development; abrupt peaks appear on the 13th and 19th days of incubation and a sharp fall just before hatching. Gill, influenced by the work of Aron ('22) and that of Potvin and Aron ('27), suggests that the sudden increase in liver glycogen during the 13th day of development is due to the appearance of insulin in the embryo.

Adrenalin was found to have no effect on the hepatic cells of cultures *in vitro*. However, injection of adrenalin into the egg on the 11th, 14th, and 18th day of incubation resulted in a marked fall in liver glycogen. Muscle glycogen, on the contrary, showed no response to adrenalin until the 18th day of incubation, when it reacted with a marked reduction. Gill consequently concluded that the enzyme system through which the adrenalin is effective is incomplete in the young embryo.

Zwilling ('48) induced hypoglycemia in chick embryos 5 to 12 days old by injecting small amounts of insulin. The hypoglycemia persisted until the 12th day of incubation, at which time it disappeared. Since it has been reported that the liver glycogen declines on the 13th day (Sakuragi, '17; Idzumi, '24; and Murray, '25), it would seem that the hepatic cells reach functional maturity and are capable of destroying the insulin, neutralizing its effect on the blood sugar.

From the above, it is difficult to draw any conclusion as to embryonic carbohydrate metabolism. Glycogen seems to be stored rapidly in the liver, as well as in the other tissues of the embryo, but the reason for this storage is still unknown. There is no agreement as to whether the embryo uses only glucose or is able to break down glycogen. In embryonic heart and skeletal muscle, glycogen was found to be present during the early stages of development, but its exact concentration and its relationship to the appearance and disappearance of spontaneous activity has not been investigated.

MATERIALS AND METHODS

The eggs used in the present studies were of several breeds: Barred Rock, Rhode Island, White Leghorn, and Plymouth Rock. Glycogen determinations were made in samples of the yolk and white of unincubated eggs, and in various tissues of embryos from three days of incubation to the time of hatching.

Embryos three to 4 days old were pooled in batches of 4 to 5. They were removed from the eggs, separated completely from the chorio-allantoic membrane, and placed in a Petri dish. The hearts were removed with the aid of a dissecting microscope and extracted separately from the remainder of the embryos.

After the 7th day of incubation, glycogen determinations were made daily. Four to 20 embryos were analyzed for each day of development. Of each of these embryos 4 extracts were made: one of the liver, one of the heart, and two of skeletal muscle. The tissue to be extracted was removed carefully with the aid of a dissecting microscope. Skeletal muscle was taken from the lower extremities, care being taken to remove completely the overlying skin and to free it from the skeleton. The time required for the preparation of the 4 samples for extraction, including the weighing on a Roller-Smith torsion precision balance, was three to 4 minutes. The amounts used in the analysis varied from 20 to 450 mg.

The glycogen of the tissue was isolated by an adaptation of the Good, Kramer, and Somogyi ('33–'37) modification of the classical Pflueger's method ('03).

In most of the samples the extracted glycogen was first hydrolyzed to glucose and then assayed, one aliquot by the copper reduction method of Folin and Wu ('17 and '20) and another by the Dreywood-Durham anthrone method ('46 and '50). In a few instances, the anthrone method was applied directly without previous hydrolysis with identical results as after hydrolysis.

Hydrolysis of glycogen to glucose was effected by the addition of 4 ml of 10% hydrochloric acid and heating in a boiling water bath for 4 hours. Colorimetric assay was made with the Evelyn photoelectric colorimeter.

The results obtained with the two methods, the copper reduction and the anthrone, differ appreciably, as will be seen in table 1. The latter ranged from 20 to 50% higher than the former. The reason for this difference is not known, but it is probable that the Folin-Wu method is not sensitive enough for the small amounts of glycogen in embryonic tissue; consequently, only the results obtained with the anthrone method will be discussed.

RESULTS

In fresh fertilized eggs the glycogen content is relatively low. The total glycogen content of the white is almost equal to that of the yolk, although the concentration is lower in the former. The mean value for the total content of the whole egg was found to be 2.7075 mg.

The youngest embryos used in the glycogen studies were three days old. The mean body weight of each embryo after removal of the heart was approximately 33 mg, and the glycogen content of each averaged 0.0043 mg or 0.013%. The heart weighed 1.45 mg on the average, contained almost as much glycogen as the rest of the embryo: 0.0036 mg, or 0.249%.

TABLE 1

Average glycogen content of embryonic tissues (grams per 100 gm of fresh tissue)

DAYS INCUBATED	LIVER		SKELETAL MUSCLE		MYOCARDIUM	
	Folin-Wu method	Anthrone method	Folin-Wu method	Anthrone method	Folin-Wu method	Anthrone method
3	. .	. .	0.0044 [1]	0.0131 [1]	0.1248	0.2492
4	. .	. .	0.0084 [1]	0.0185 [1]	0.1213	0.2263
8	0.1701	0.5543	0.0044	0.0654	0.3786	1.1929
9	0.1100	0.2554	0.0353	0.0489	0.5566	1.1539
10	0.3475	0.7565	0.0315	0.0778	0.5505	1.6234
11	0.0276	0.5420	0.0257	0.0825	0.4614	1.1827
12	0.2986	1.1558	0.0285	0.0976	0.7098	1.2767
13	0.4132	0.5615	0.0561	0.1554	1.1150	1.0270
14	0.5306	0.6247	0.0757	0.0941	0.5154	0.6880
15	0.3228	1.0880	0.0409	0.0915	0.4700	0.8775
16	1.2809	1.6475	0.0858	0.2165	0.7202	1.2782
17	0.8787	1.5161	0.1182	0.2433	0.4400	0.8813
18	1.3042	2.8904	0.1344	0.3586	0.2621	0.5412
19	0.4769	1.5280	0.0805	0.3232	0.1844	0.7701
20	1.2048	1.2364	0.1272	0.3344	0.4391	0.9861
21	1.1282	1.7722	0.1810	0.3326	0.3103	0.5461

[1] Entire body except myocardium.

In 4 day old embryos, the average body weight was 49.4 mg and the average heart weight, 5.1 mg. The total body glycogen (except for the heart) averaged 0.0082 mg, or 0.018%; the heart glycogen, 0.0115 mg, or 0.226%.

The values for liver, heart, and skeletal muscle glycogen of embryos from 8 days until hatching are presented in table 1. In figure 1 the glycogen concentrations of the three tissues are plotted against the days of incubation.

DISCUSSION

Embryonic tissues seem to produce and store glycogen from the earliest stages of development. In three day old embryos, the concentration of polysaccharide is already considerably higher than in the egg (white or yolk). Moreover, the glycogen is not evenly distributed; its concentration in the most active tissue, the heart, is more than 20 times greater than in other tissues analyzed. This uneven distribution of glycogen, with preferential storage in the pulsating myocardium, would not take place if embryonic tissue were unable to utilize the polysaccharide, as has been postulated by Needham and Nowinski ('37). Also, against the last hypothesis is the fact that the glycogen concentration in a given organ does not remain uniform, but varies from one stage to another, increasing one day and decreasing on the next. (These findings were recently confirmed in analyses of a new series of embryos.) The fluctuations observed

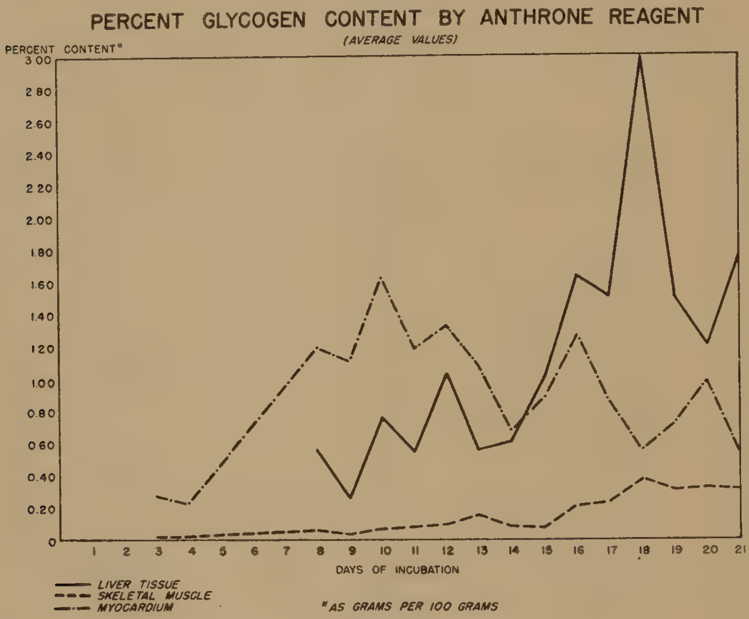


Figure 1

in the glycogen content of the various tissues suggest that glycogen is continuously formed and destroyed.

This raises the question of whether the embryo possesses some regulatory mechanism for the distribution of glycogen among the various body tissues, or whether an increase in the polysaccharide concentration of one organ automatically causes a depletion in another tissue or organ. The last hypothesis is not supported by the data presented in this paper, since on certain days of incubation an overall increase or decrease of the glycogen concentration was observed in all three of the tissues studied. However, before any definite conclusion can be drawn, data on other tissues are necessary, particularly on nervous tissue, lungs, the smooth muscle of the digestive canal, the chorio-allantoic membrane, etc., all of which also contain glycogen.

According to Aron ('22), Potvin and Aron ('27), and Villamil (1892), insulin appears and begins to influence the carbohydrate metabolic mechanism in the chick embryo on the 11th–12th day of incubation. The results described in the present paper are not in agreement with this point of view. No drastic change was observed in the glycogen storage of the three tissues studied in 11 or 12 day old embryos. The heart is the only tissue of the three in which the glycogen concentration reaches its highest level on the 11th–13th days of incubation. Glycogen is present in liver from the moment of its appearance, and increases gradually, although not rectilinearly, up to the 18th day of incubation. According to Potvin and Aron, the decrease in liver glycogen observed on the 11th day of incubation is caused by the initial production of insulin; however, decreases in this value were also observed on the 9th day, the 13th–14th days, the 17th day, and on the last two days before hatching.

In skeletal muscle, glycogen is present at a very early stage of development and increases slowly in concentration during the second week of incubation. No abrupt change was observed at the moment of appearance of the first embryonic movements, that is, on the 4th day of incubation (Kuo

Zing Yang, '32; Windle and Orr, '34). On the contrary, between the 15th and 18th day of incubation the glycogen of skeletal muscle shows a rapid and considerable increase. Its concentration on the 18th day of incubation is almost 4 times greater than on the 15th day. Curiously enough, this abrupt increase coincides with changes in muscle behavior. As was mentioned previously, the spontaneous activity which appears in tissue culture of skeletal muscle from young embryos decreases gradually in muscle of 13 to 18 day old embryos, and completely disappears in explants prepared from embryos older than 18 days. It is interesting to note that after denervation, adult muscle rapidly loses 50% of its glycogen content (Hines and Knowlton, '35).

One may conjecture that the change in glycogen content influences the behavior of embryonic muscle. Perhaps with an increase in content and with stabilization of glycogen, the embryonic muscle acquires the properties of adult muscle, and thereby loses its spontaneous activity, responding only to stimuli. More data are necessary to support this hypothesis.

SUMMARY AND CONCLUSIONS

The glycogen of liver, heart, and skeletal muscle of chick embryos in various stages of development was isolated by the classical Pfluger method and determined by the Folin-Wu copper reduction method and by Dreywood's anthrone method, as modified by Durham.

In the liver, glycogen is found as early as the 7th or 8th day of incubation, and increases gradually up to the 18th day of incubation. The increase is step-wise, presenting depressions on the 9th, 11th, 13th–14th, 17th, and 19th–20th days.

In the heart, on the third day of incubation, the glycogen concentration is more than 20 times greater than in the other tissues. Myocardial glycogen increases rapidly up to the 11th–13th day of incubation, and then decreases slightly during the last week before hatching.

In skeletal muscle, the glycogen concentration increases slowly during the first two weeks of incubation. An abrupt increases occurs between the 15th and 18th days of incubation, with little further variation before hatching.

Since fresh eggs are relatively poor in glycogen, and in view of the daily variations in the glycogen concentration in the three tissues studied, it is suggested that the embryo both synthesizes and uses glycogen.

An inverse relationship was found to exist between the glycogen concentration and the spontaneous contractibility of skeletal muscle. From this it would appear that glycogen storage plays an important part in the transformation of embryonic muscle, which is spontaneously active, into adult muscle, which responds only to stimuli.

LITERATURE CITED

ARON, M. 1922 Le glycogen du foie embryonnaire. Determinisme de sa formation. Bull. de la Soc. de Chimie Biol., *4:* 209–222.

DONHOFFER, C. 1933 Studies on the carbohydrate metabolism of the chick embryo. Biochem. J., *27:* 806–817.

DREYWOOD, R. 1946 Qualitative test for carbohydrate material. Ind. and Eng. Chem. (Anal. Ed.), *18:* 499–500

DURHAM, W. F., W. L. BLOOM, G. T. LEWIS AND E. E. MANDEL 1950 Rapid measurement of carbohydrate in blood. Pub. Health Rep., *65:* 670–674.

FOLIN, O., AND H. WU 1917 A system of blood analysis. J. Biol. Chem., *38:* 81–88.

——— 1920 A system of blood analysis. Supplement I. A simplified and improved method for determination of sugar. J. Biol. Chem., *41:* 367–374.

GILL, P. M. 1938 The effect of adrenaline on embryonic chick glycogen in vitro as compared with effect in vivo. Biochem. J., *32:* 1792–1799.

GOOD, C. A., H. KRAMER AND M. SOMOGYI 1933 The determination of glycogen. J. Biol. Chem., *100:* 485–491.

HINES, M., AND G. C. KNOWLTON 1935 The role of the nervous system in the relation of the glycogen metabolism of skeletal muscle. Am. J. Physiol., *111:* 243–249.

IDZUMI, S. 1924 Mitteilungen a. d. med. Facultat Univ. Tokio. Needham's Chemical Embryology.

KUO ZING YANG 1932 Ontogeny of embryonic behavior in aves. I. The chronology and general nature of the behavior of the chick embryo. J. Exp. Zool., *61:* 395–430.

MURRAY, H. A. 1925 Physiological ontogeny. A. Chicken embryos. II. Catabolism. Chemical changes in fertile eggs during incubation. Selection of standard conditions. J. Gen. Physiol., *9:* 1–48.

NEEDHAM, J. 1931 Chemical Embryology. Cambridge University Press.

NEEDHAM, J., AND W. W. NOWINSKI 1937 Intermediary carbohydrate metabolism in embryonic life. I. General aspect of anaerobic glycolysis. Biochem. J., *31:* 1165–1185.

NEEDHAM, J., W. W. NOWINSKI, M. C. DIXON AND R. P. COOK 1936 Mechanism of carbohydrate breakdown in early embryonic development. Nature, *138:* 462–463.

———— 1937 Intermediary carbohydrate metabolism in embryonic life. II. The formation and removal of pyruvic acid. III. The Pasteur effect and the Meyerhof cycle. IV. The distribution of acid soluble phosphorus. Biochem. J., *31:* 1185–1209.

NOVIKOFF, A. B., V R. POTTER AND G. A. LEPAGE 1948 Phosphorylating glycolysis in the early chick embryo. J. Biol. Chem., *173:* 239–252.

PFLUEGER, E. 1903 Die Entdeckung des Glycogen. Arch. f. d. ges. Physiol., *96:* 1–398.

POTVIN, R., AND M. ARON 1927 Recherches sur l'evolution embryonnaire des ilots pancreatiques endocrines chez le Poulet. C. R. Soc. Biol., *96:* 267–268.

SAKURAGI, K. 1917 Tokio Med. Soc. Needham's Chemical Embryology.

SOMOGYI, M. 1937 A reagent for the copper-iodometric determination of very small amounts of sugar. J. Biol. Chem., *117:* 771–776.

SZEPSENWOL, J. 1946 A comparison of growth, differentiation, activity, and action currents of heart and skeletal muscle in tissue culture. Anat. Rec., *95:* 125–146.

VILLAMIL, M. F. 1942 Citogenesis del pancreas exo y endocrina en embriones del pollo. Rev. de la Soc. Argentina de Biol., *18:* 416–428.

VLADIMIROV, G. E., AND M. I. DANILINA 1930 Studies in embryological chemistry and physiology. IV. Accumulation of glycogen in the organism of the developing chick embryo. Biochem. Z., *224:* 69–78.

WINDLE, W. F., AND D. W ORR 1924 The development of behavior in chick embryos; spinal cord structure correlated with early somatic activity. J. Comp. Neur., *60:* 287–307.

ZWILLING, E. 1948 Insulin hypoglycemia in chick embryos. Proc. Soc. Exp. Biol. and Med., *67:* 192–193.

THE ROLE OF A THIRD GONADOTROPHIC HORMONE IN THE MECHANISM OF ANDROGEN SECRETION IN CHICKEN TESTES[1]

A. V. NALBANDOV
Department of Animal Science, University of Illinois
R. K. MEYER AND W. H. McSHAN
Department of Zoology, University of Wisconsin. Madison

THIRTEEN FIGURES

In earlier papers the present authors ('46) and Nalbandov and Baum ('48) showed that hypophysectomized or stilbestrol-inhibited male chickens are sensitive test animals for gonadotrophic hormones. It was shown in these reports that either surgically or physiologically hypophysectomized males can be used to distinguish between pure follicle stimulating hormone (FSH) preparations and those containing predominantly luteinizing hormone (LH). FSH gave testicular growth and an increase in tubule diameter without comb growth; FSH contaminated with LH produced an increase in testis size along with a reddening of the skin of the head region if the contamination with LH was slight, or actually comb growth if the LH was present in more than traces. The increases in comb size produced with pure LH were substantial and, depending on the dose of LH given, amounted to a 2- to 5-fold increase over the atrophic combs of the untreated controls.

---

[1] This study was begun as a cooperative project between the two institutions involved. The technical assistance of Miss G. J. Baum is gratefully acknowledged. This investigation was supported by grants from the Committee for Research in Problems of Sex, National Research Council and from the Wisconsin Alumni Research Foundation. We wish to thank Dr. R. W. Bates (E. R. Squibb and Sons), Schering Corporation (Bloomfield, N. J.), and Wick and Fry, Inc., for generous gifts of prolactin, desoxycorticosterone acetate and stilbestrol pellets.

In general, gonadotrophic hormones injected into hypophysectomized mammals stimulate the gonads and maintain them and the accessories stimulated by their secretions at approximately their normal weights for prolonged periods of time. Thus the seminal vesicles and the prostate of hypophysectomized male rats may be maintained in good morphological and histological condition for several months by the injection of either crude or purified gonadotrophic hormones containing LH. Hypophysectomized cocks offer an interesting exception to this rule because mammalian gonadotrophic hormones injected into them can increase and maintain the size of the testis but are not able to cause sustained secretion of androgen. Thus, hypophysectomized cocks injected with gonadotrophic hormones of mammalian origin will respond with comb growth for a constant and highly predictable interval of 10 to 12 days, after which the combs regress in spite of continued injections of the hormone. It became of interest to determine the cause of this discontinuation of comb growth and its underlying discontinuation of androgen secretion by the testes.

Animals and hormones used

Male chickens of the White Leghorn variety were used in all experiments. Two methods of depriving them of their own gonadotrophic hormones were used: hypophysectomy and stilbestrol inhibition. Hypophysectomy or the implantation of a stilbestrol pellet were performed when the males were 60 days old and weighed about 600 gm. For both treatments it required 10 to 14 days for the comb to regress completely to its minimum (capon) size, at which time it also became pale, dry and shriveled. The stilbestrol-treated cocks required no special environment. The hypophysectomized cocks survived the operation well if they were kept in an environmental temperature of about 80°F. and without sudden changes in the temperature. Equally important for their survival was the fact that they were given an opportunity to eat during the night, thus preventing hypoglycemia resulting from the long fast during darkness. A time clock was set to give them one hour of light and thus an opportunity for feeding at 10 P.M. and 2 A.M. This was particularly important during the first two to three weeks after hypophysectomy and accounted for the high mortality of hypophysectomized chicken observed in earlier work (Nalbandov and Card, '43). These precautions reduced the mortality following hypophysectomy to less than 5%.

The mammalian gonadotrophic hormones used were purified preparations of FSH and of LH, and an unfractionated preparation of sheep pituitary powder (ISAP) made by the methods of McShan and Meyer ('40, '43, '46); an acetone-dried powder of sheep anterior pituitary glands (SLP); and pregnant mare serum (PMS) (Upjohn–Gonadogen).

FSH, LH, and ISAP were injected subcutaneously at the rate of 5 gm — equivalent of the acetone dried anterior lobe powder per male per 8 days. The SLP was given at the rate of 200 mg per male per 8 days and the PMS at the rate of 25 and 50 Cartland-Nelson units per male per 8 days.

Whole unfractionated chicken pituitary powder was also used in some of the experiments. Chicken pituitaries were obtained from heads which were shipped frozen to this laboratory from large poultry dressing establishments. The glands were removed from the still-frozen heads as rapidly as possible and kept frozen until needed. They were crushed between glass plates and air-dried before grinding with pestle and mortar. Chicken pituitary powder was suspended in water and injected subcutaneously at the rate of 40 mg equivalent of wet gland per chicken per day.

The effectiveness of the different treatments was judged by the changes produced in the comb size of the injected birds. The results are reported as relative "comb area," (the product of the maximum length of the comb and its height directly above the eye) taking comb area on the day on which injections were started as 100. Comb measurements were begun at hypophysectomy or estrogen implantation and were taken on alternate days during the whole experiment, but to save space the changes in comb area are reported at longer intervals beginning with the first day of injection.

As mentioned earlier, 10 to 14 days after hypophysectomy or stilbestrol implantation were required for the comb to regress to capon size. Hence all injections were started on the 16th day after treatment or hypophysectomy. In the three experiments discussed all the birds in each group were initially injected with the same gonadotrophic hormone and for this reason the changes in their comb areas were averaged. Each point from 0 to 20 or 24 days represents 8, 12 or 16 birds depending on the number of hormones which were to be used following the initial stimulation. In all treatments following the initial stimulation 4 birds are represented at each point. Four hypophysectomized males remained untreated and were used as controls.

In a separate experiment conducted in the same manner as the one just described, males were killed at various intervals during the experiment to obtain data on the changes in testes weights and testes histology in relationship to comb growth. Similar information was obtained from the testes of a few males which became moribund during other experiments.

RESULTS

The general design of the experiments is apparent from figures 1, 2 and 3. Three types of hormones were used as initial gonad stimulators: a crude pituitary preparation (SLP) containing LH, FSH as well as the non-gonadotrophic pituitary hormones (fig. 1), a crude LH (fig. 2) and pregnant mare serum (PMS) (fig. 3). With daily injection of these hormones, comb growth lasted for only 8 to 12 days. Injections were continued for 20 days in case of SLP and LH and for 24 days in the case of PMS. After these periods it was quite apparent that regression in comb size was irreversible, and various other hormones were substituted.

Experiment 1 (fig. 1). After the crude sheep pituitary powder (SLP) had failed to maintain comb growth, 4 different hormones were substituted for it. LH was able to reinitiate androgen secretion, and hence comb growth, for about 8 days causing an increase in comb area of 10%. In spite of LH injection continued for 14 more days, the comb again regressed to capon size. When PMS was substituted for LH at the end of that period, it was not able to reinitiate comb growth.

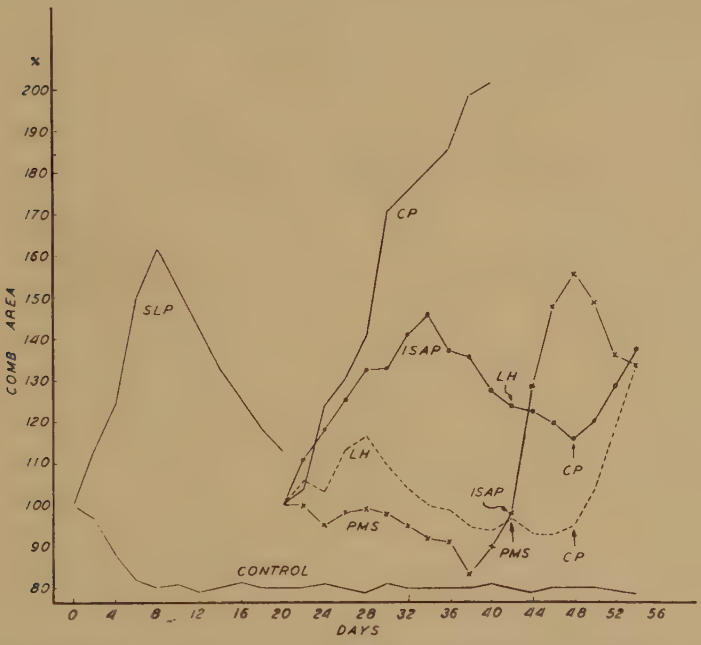


Fig. 1 Response of combs of hypophysectomized male chickens to various gonadotrophic hormones. SLP, sheep anterior pituitary powder; ISAP, unfractionated, partly purified gonadotrophic hormone (sheep); LH, purified luteinizing hormone (sheep); PMS, pregnant mare serum; CP, chicken pituitary powder.

Another hormone substituted for SLP was the partially purified ISAP which contains predominantly FSH and LH. It caused a greater increase in comb area than did LH and comb growth continued for 14 days but again growth was temporary and substitution of LH after 22 days was unable to halt the decrease in comb size.

In a third group of 4 birds SLP was followed by PMS (Gonadogen). In contrast to the two pituitary preparations, PMS was unable to increase androgen secretion and comb area continued to decrease. The increase in comb area shown on the 37th day (fig. 1) is due to the death of one of the 4 birds and is statistically not significant.

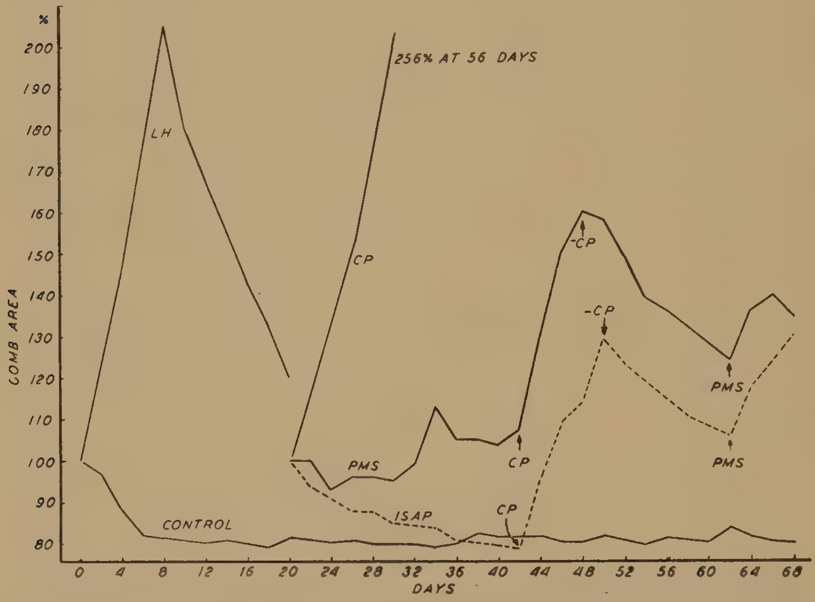


Fig. 2 Response of combs of hypophysectomized male chickens to various gonadotrophic hormones (for explanation see fig. 1).

After it became apparent that PMS would not renew androgen secretion, ISAP was injected and immediate but temporary comb growth resulted.

Experiment 2 (fig. 2). In this experiment the initial hormone used was LH. After the comb had almost returned to its initial size LH was replaced by PMS in one group of 4 birds and ISAP in another. Neither of these hormones produced significant comb enlargement.

Experiment 3 (fig. 3). In a final group of 12 birds, PMS was the starting hormone followed by SLP, LH and ISAP. All three of these preparations caused additional, though very temporary, comb growth.

In all three experiments chicken pituitary powder (CP) was supplied or withdrawn as indicated on the graphs. The discussion of the effects of this treatment will be deferred, but it should be noted that regardless of where CP was substituted for the no longer effective mammalian gonadotrophs, it produced immediate and sustained comb growths (figs. 1, 2 and 3).

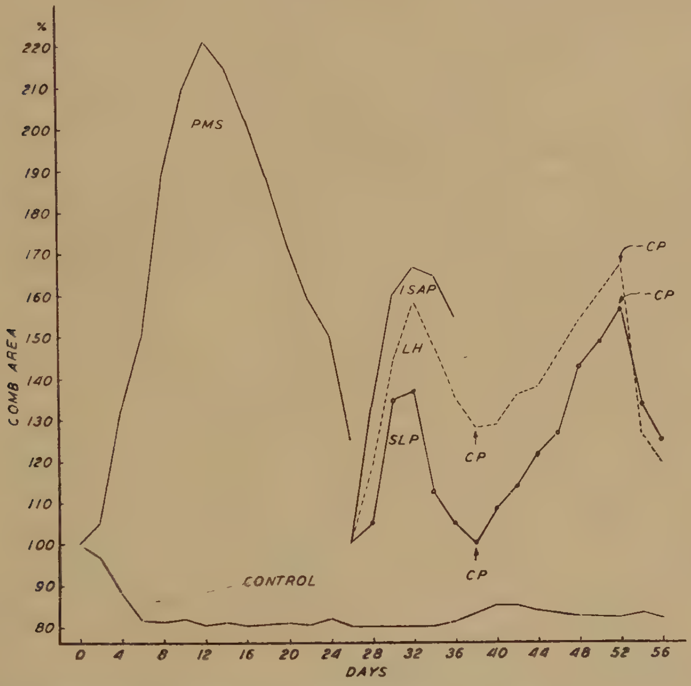


Fig. 3 Response of combs of hypophysectomized male chickens to various gonadotrophic hormones (for explanation see fig. 1).

In the light of the theory to be advanced later in this paper it is important to call attention to the observed relationship between the weight of the testes and the changes in comb size.

Hypophysectomy always results in complete regression of comb and testes. In the experiments under discussion the first stimulation with a mammalian gonadotrophic hormone the comb increased in size and so did the testes. But, as the comb underwent its first involution the testes remained enlarged and continued to grow as long as the hormones were being administered (table 1). It thus appears, that as far as the mammalian gonadotrophic hormones are concerned, there is no relationship between their ability to cause testicular growth and their ability to cause androgen secretion. The significance of this observation will be discussed later.

TABLE 1

Effects of chicken pituitary powder and PMS on comb growth and testes size of hypophysectomized cocks

NO. OF HYPOPHYSECTOMIZED MALES	TREATMENT	CHANGES IN COMB AREA (100% ON DAY INJECTIONS BEGAN)							AVER. TESTES WT. AT END OF EXPERIMENT
		At operation	Number of days: after first injection						
			0	6	12	18	24	36	
									mg
4	None	206.3	100	94.3	91.1	90.6	89.5	90.2	99
4	Chicken pituitary	176.5	100	134.1	158.0	188.0	225.5	291.1	352
5	PMS	156.9	100	118.9	134.5	127.1	107.3	105.5	463

It is also important to call attention to one other finding which is not documented in this paper but which is of importance for support of the theory to be presented. Testes did not regain their ability to respond to the hormone which was used for the original stimulation and exhaustion. Thus even after rest periods of three to 6 months combs of males treated initially with LH showed no response to any mammalian gonadotrophic hormone. Thus males treated initially with LH, even after rest periods of three to 6 months did not respond by comb growth if at the end of these periods they were again treated with any of the mammalian gonadotrophic hormones. In general the response to mammalian gonadotrophins was exactly the same after rest periods as when the post-treatment is given immediately following the initial stimulus.

Interpretation of results

In general the results show three broad patterns:

1. Injections of mammalian gonadotrophic hormones into hypophysectomized cocks caused androgen secretion, and hence comb growth, for a very limited and rather stable period of 10 to 12 days, followed by complete regression of the comb in spite of continued treatment (figs. 1, 2 and 3).

2. A second but less intense wave of androgen secretion, and comb growth, was produced with other mammalian gonadotrophic substances if the initial stimulation was caused by a crude, unfractionated pituitary preparation (SLP or ISAP) or by PMS, but not if the original stimulation was caused by a highly purified pituitary LH (compare figs. 1, 2 and 3).

3. In all instances chicken pituitary powder was capable of producing sustained comb growth regardless of the preceding treatment.

4. The mammalian hormones used caused temporary comb growth but were able to increase testicular weight significantly and to maintain it in spite of the observed fluctuations in comb size (table 1).

Several possible plausible explanations of these results were subjected to experimental tests.

Refractiveness of the comb. Even though it is apparent from the graphs that the comb did not lose its ability to respond to androgen, an experiment was performed in which androgen was injected both at the time when the comb had reached its peak after gonadotrophic stimulation and was due to start to regress, and at the time when regression was already well underway or had been completed. In the former case injection of androgen prevented regression; in the latter two cases comb growth was reinitiated, demonstrating that the comb was still capable to respond to androgen.

Tests for antihormones. The next possibility considered was that antihormones were formed which counteracted the injected mammalian gonadotrophs. Two considerations speak against this possibility. First, the refractory period sets in within 10 to 12 days after the initial injection, an unusually short period for the appearance of antihormones. Furthermore the refractory period sets in almost simultaneously for all birds, deviating not more than one day from the average time. Antihormones also would be expected to disappear within a few months after the injections of hormones were discontinued. However, it was found that a rest period as long as 6 months following an initial stimulation with SLP, PMS, or LH, did not restore the ability of the testes to secrete androgen.

The antihormone theory was ruled out completely by demonstrating that no antibodies were formed following the injection of an ISAP preparation into three intact male chickens for 33 days and by sampling their blood serum at 15 and 33 days. These sera were tested with the same ISAP preparation in various concentrations on immature chicks, but no antihormones were found in the serum at the two periods at which samples were taken. It is also interesting that, for the three males into which the ISAP was injected in the attempt to produce antihormones, the combs were constantly and continuously larger than the combs of the three uninjected controls and did not show the refractory period always observed in hypophysectomized males injected with gonadotrophs (tables 2 and 3).

Tests for missing hormone. Next it was considered that hormones other than those injected may be necessary for the continuous production of androgen by the chicken testis. To examine this possibility, the following hormones were added to the gonadotrophs used: thyroxin, iodinated thyroprotein, traces of androgen insufficient by themselves to cause pronounced comb growth, estrogen, prolactin, progestin, FSH, and desoxycorticosterone acetate. Reasons for including most of these hormones are obvious, but some were added primarily for the sake of completeness. Some of the most logical contenders for the role of the ''missing hormone,'' such as the thyroidal preparations and estrogen were tried at several levels, including traces. None of these hormones caused sustained comb growth when given in combination with a mammalian gonadotroph and, in all combinations tried, the decline in comb size began at the usual time after the initial injection of the gonadotrophic hormone. Since all of these experiments gave negative results their documentation is omitted.

Histology of testes. It was mentioned earlier that testes weights did not parallel changes in comb size (table 1). Thus cocks injected with PMS for 30 days responded by initial comb growth followed by complete atrophy of the combs, in spite of continued injection with PMS. During this same time the testes did not regress in size and were in fact heavier at the time when the combs had regressed completely than they were at the time of their maximum stimulation. The same relationship between testes weight and comb size was also found for the other gonadotrophic hormones used.

TABLE 2

Effect of unfractionated sheep pituiary extract on normal males injected for 33 days

TREATMENT	DURATION OF EXPERIMENT	NUMBER OF ANIMALS	COMB GROWTH AREA CM^2		TESTES WEIGHT
			Start	Finish	
	days				*gm*
Control	33	3	51.1	89.1	11.75
ISAP (P32B)	33	3	49.5	110.2	18.14

TABLE 3

Testes weight of chicks injected with ISAP in combination with sera to be tested for antihormone

GROUP NO.	I	II	III	IV	V	VI	VII
Total dose of hormone (ISAP) per chick	3 gm equiv. in 9 inj.					0	0
Total dose of serum per chick (cm^3)	0.13	0.25	0.50	1.00	0	1.00	0
Average testes weights of 3 chicks (gm)	.041	.057	.042	.047	.043	.011	.008

It was further observed that in certain cases (to be discussed in detail later) spermatogenesis was taking place in spite of the fact that the combs of such males were completely atrophied.

These findings suggested the possibility that the testes respond differently to the tubule-stimulating hormones than to LH. The breakdown may occur only in the androgen-secreting mechanism; the testes as a whole keeping their ability to continue to respond to gonadotrophic hormones by tubule growth.

For these reasons, a study of the histology of the testes, particularly of the interstitial tissue, was made. It was found possible to ascertain consistently on the basis of the appearance of the Leydig cells whether the combs of hypophysectomized males treated with mammalian gonadotrophins were in the growth, regression, or atrophy phase. The main differences in the cells were as follows: during the growth phase, the majority of the Leydig cells were vacuolated (fig. 6); during the decline in comb growth following the initial stimulation with gonadotrophic hormone, the majority of the cells were contracted (distinct from the vacuolated type), but not pycnotic (fig. 7). Finally, after the second stimulation with a gonadotrophin, the number of vacuolated cells during the growth phase of the comb was much smaller than during the first growth phase and the majority of the Leydig cells were pycnotic with a complete absence of both vacuolated cells and the contracted non-vacuolated cells (fig. 8).

The mechanism of androgen secretion. Differences in the appearance of the Leydig cells at the various stages of comb growth and atrophy, together with the observed responses of the combs to gonadotrophic hormones, suggested that androgen secretion in the cock is a two-step process (fig. 4). In this process, secretory Leydig cells of two types, A and B, arise *de novo* from non-secretory precursor cells, both types becoming non-secretory and ''exhausted'' pycnotic cells after secretion caused by stimulation by mammalian gonadotrophic hormones. Cell types A and B and the pycnotic cells in ''exhausted'' testes can be identified histologically (figs. 5 to 10).

On these assumptions and observations the following interpretation of the observed results became possible:

1. Mammalian, LH-containing hormones can act only on cell types A and B, and can cause type B to secrete androgen. After cell B has been stimulated once, it is incapable of secreting androgen becoming exhausted and pycnotic. This would account for the fact that androgen secretion and hence comb growth are temporary after the testes are stimulated with mammalian gonadotrophic hormone.

2. This accords with the fact that comb growth and hence androgen secretion are continuous in the intact cock. Avian pituitaries contain a hormone, not found in mammalian gonadotrophs, which transforms the non-secretory precursor Leydig cells into cell types A and B.

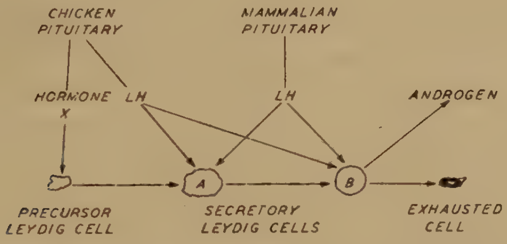


Fig. 4 Diagramatic representation of the mechanism of androgen secretion in cocks; the succession of secretory and non-secretory Leydig cells as they are affected by mammalian and avian LH. For details see text.

Acting on these assumptions a series of experiments was begun in which chicken pituitaries were used. If the assumptions made were correct, chicken pituitaries must have the following properties:

(a) They must be able to cause continuous androgen secretion and comb growth in hypophysectomized males given only chicken pituitary injection.

(b) Testes exhausted with several mammalian LH-containing gonadotrophs must resume androgen secretion when subjected to chicken pituitary stimulation. In cases in which testes were exhausted with mammalian gonadotrophic preparations, a short time treatment with chicken pituitary should restore the ability of testes to respond to the formerly ineffective mammalian gonadotrophs.

All of these conditions were met (figs. 1, 2 and 3). Because of the difficulty of obtaining chicken pituitaries it was not always possible to make the injections last for the whole duration of the experiment but in all cases the treatment was continued for a period considered adequate for proof. If chicken pituitary powder was injected as the starting material, growth continued for as long as the injections were given (56 days) and the size of the comb reached during that time was 256% (fig. 2). If chicken pituitary followed a mammalian hormone (SLP, fig. 1), it reinitiated comb growth and maintained it for as long as it was injected. After testes "exhausted" with LH, PMS or ISAP, had been "primed" with chicken pituitary powder, a gonadotrophic hormone (PMS) which had previously been unable to cause androgen secretion was again capable of doing so, even after an interval of 12 days between the last injection of chicken pituitary powder and the PMS (fig. 2).

One further experiment adds to this interpretation. Intact cocks were implanted with estrogen pellets capable of inhibiting the animal's own pituitary to such an extent that a complete regression of comb and testes resulted. Following this treatment, they were injected with a variety of mammalian gonadotrophic hormones and responded in the same manner as did the surgically hypophysectomized males. After no further androgen secretion could be caused by any sequence of combination of mammalian gonadotrophs, the estrogen pellets were removed and the animal's own pituitary was allowed to begin to come back to normal secretory activity. At the time when the combs first showed signs of commencing growth (an average increase of not more than 10% in area), the birds were reimplanted with estrogen and their pituitaries were again completely inhibited. When such animals were then again subjected to a mammalian gonadotroph, they responded in the typical manner by temporary comb growth of 10 to 12 days duration. This is interpreted to mean that during the short period during which the animals were not under the inhibiting influence of estrogen, their own pituitaries had secreted enough of the hormone necessary for the differentiation of precursor cells into a new crop of secretory Leydig cells.

DISCUSSION

The results presented earlier in this paper argue for the assumption that androgen secretion in the chicken is controlled not only by LH, as it seems to be in the mammal, but by a third gonadotrophic hormone which has properties distinct from those of mammalian LH. This third hormone seems to be a true interstitial cell stimulating hormone, an avian ICSH, in that its main role is to cause the metamorphosis of non-secretory Leydig cells (precursor cells) into secretory Leydig cells. On the evidence presented it is not possible to rule out the possibility that avian LH is an entity with two distinct properties, one affecting cell morphology, the other stimulating Leydig cells to secrete androgen. Whether we are dealing with a third gonadotrophic hormone or with a conventional LH with dual properties cannot be investigated until chicken pituitaries are fractionated and purified.

Some indirect evidence which argues for the assumption that there is a third avian gonadotrophic hormone, is based on three cases of hypophysectomized chickens, one treated with an unfractionated sheep pituitary preparation (ISAP) and two with PMS. In the first, normal spermatogenesis was found in some of the testis tubules (fig. 1), and the other two showed spermatogenesis also, though abnormal in some respects. In all three cases the combs were completely atrophic at the time when spermatogenesis was in progress, i.e., at the time of autopsy. This indicates that no androgen was being or had been secreted for some time before autopsy; this was further corroborated by the testis histology which showed only pycnotic, non-secretory Leydig cells commonly associated with regressed combs. In earlier studies it has been found that neither mammalian FSH nor LH alone is capable of causing spermatogenesis in the bird, but together are able to do so. In these three cases then, the conditions were satisfactory for spermatogenesis caused by mammalian gonadotrophic hormones, but were not satisfactory for androgen secretion and comb growth. It follows that LH was present, but that an essential for continuous secretion was lacking. This argues that there is avian ICSH which is an independent hormone rather than that LH has dual properties.

One difficulty encountered in this study lies in the fact that two successive periods of androgen secretion were produced if the first hormone used after hypophysectomy was a natural mixture of FSH and LH (figs. 1 and 3). However, only one period of androgen secretion resulted if the initial trophic hormone was purified LH (fig. 2). No satisfactory explanation of this observation is available. The histology of the testes treated with these two classes of hormones suggests that pure LH is much more effective in exhausting the available secretory Leydig cells, all cells remaining in the interstitium after that hormone being pycnotic, while after the crude gonadotrophins this is not the case. The reasons for these differential effects are not clear, although it is obvious that the natural FSH-LH mixtures do not exhaust all the available Leydig cells. Studies on these effects are being continued.

SUMMARY

1. Combs atrophied as the result of hypophysectomy of male chickens can be caused to grow for 10 to 12 days by treatment with mammalian, LH-containing gonadotrophs, but regression to preinjection size follows in spite of continued injection of such hormones.

2. This discontinuation of comb growth is not the result of refractiveness of the comb tissue itself nor of the formation of antihormones, nor is it due to lack of other pituitary, thyroid, or gonadal hormones.

3. In hyophysectomized males, continuous comb growth can be produced by the injection of chicken pituitary powder.

Testes which fail to secrete androgen in response to mammalian LH-containing hormones, may be caused to do so continuously if chicken pituitary powder is substituted for the mammalian hormone.

4. Testes which fail to respond to mammalian gonadotrophs by androgen secretion continue to respond to these hormones by size increases (tubule growth) and, in some cases, by spermatogenesis.

5. The theory is proposed and supported that avian pituitaries either contain a third gonadotrophic hormone (avian ICSH) or have an LH possessing different properties from mammalian LH.

6. Testes histology supports the contention that avian ICSH (or a part of avian LH), causes the metamorphosis of non-secretory (precursor) cells into secretory Leydig cells which secrete androgen when stimulated by mammalian or avian LH.

7. Mammalian pituitary hormones do not contain the hormone necessary for the formation of new Leydig cells and thus are able to cause androgen secretion only so long as already formed secretory Leydig cells are still available. As these become exhausted androgen secretion stops and thus accounts for the discontinuous comb growth observed in hypophysectomized chickens treated with mammalian gonadotrophs.

LITERATURE CITED

McShan, W. H., and R. K. Meyer 1940 The preparation and properties of pituitary follicle-stimulating fractions made by trypsin digestion. J. Biol. Chem., *135:* 473–482.

——— 1946 Further purification of the follicle-stimulating hormone and its effect in normal hypophysectomized rats. Proc. Soc. Exp. Biol. Med., *61:* 57–62.

Nalbandov, A. V., and G. J. Baum 1948 The use of stilbestrol inhibited males as test animals for gonadotrophic hormones. Endocrinol., *43:* 371–379.

Nalbandov, A. V., and L. E. Card 1943 Effect of hypophysectomy of growing chicks. J. Exp. Zool., *94:* 387–413.

Nalbandov, A. V., R. K. Meyer and W. H. McShan 1946 Effect of purified gonadotropes on the androgen secreting ability of testes of hypophysectomized cocks. Endocrinol., *39:* 91–104.

PLATE 1

EXPLANATION OF FIGURES

Figures 5 to 11 show interstitial tissue and portions of seminiferous tubules of testes from untreated and treated hypophysectomized male chickens. Magnifications in figures 5 through 9, and 11 are × 450.

5 Untreated, hypophysectomized; comb regressed.

6 Six days after injection of PMS; comb growing rapidly. Compare Leydig cells with figures 5 and 6; germinal epithelium with 5.

7 After 20 days of continuous LH treatment; comb completely regressed. Note shape and size of Leydig cells; some of these may be ''precursor'' cells.

8 After 20 days of PMS treatment (first wave of androgen secretion) followed by 12 days of SLP treatment (second wave of androgen secretion); comb completely regressed. Note the presence of a few ''exhausted'' Leydig cells.

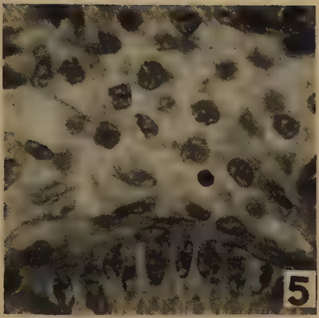
9 After 10 days of treatment with chicken pituitary powder; comb growing rapidly. Vacuolated Leydig cells of types A and B are present.

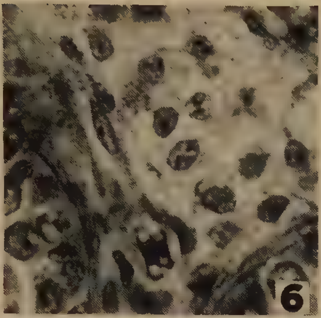
10 Same as figure 5. × 1200.

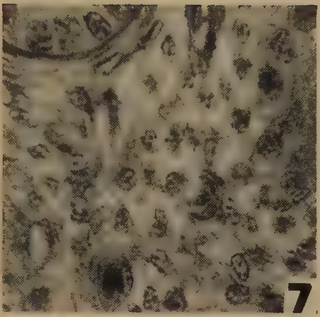
11 Spermatogenesis in absence of androgen secretion following ISAP treatment; comb completely regressed. Note inactive neighboring tubule.

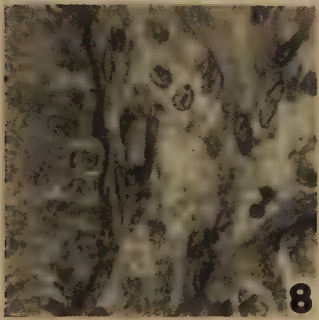
12 Appearance of untreated hypophysectomized brown Leghorn male (used in earlier experiments).

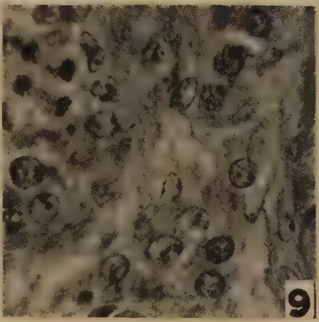
13 Male shown in figure 12 after treatment with LH for 10 days. Photographed three days before regression in comb size began.

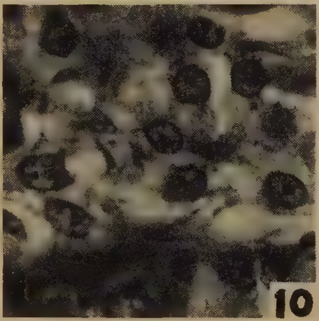

5

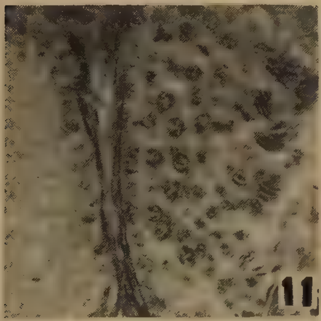

6

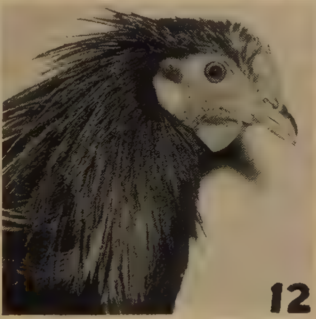

7

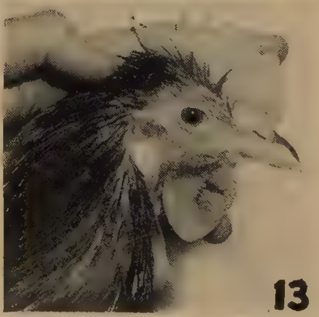

8


9


10


11


12


13

CYTOLOGICAL DISTRIBUTION OF CHOLESTEROL AND ASCORBIC ACID IN THE ADRENAL CORTEX OF THE RAT EXPOSED TO COLD

HABEEB BACCHUS
Department of Physiology, The George Washington University,
School of Medicine, Washington, D. C.

SIX FIGURES

Two very outstanding chemical characteristics of the adrenal cortex are its high content of cholesterol and ascorbic acid. There is increasing chemical evidence that both these substances are involved in the metabolic and secretory activity of the adrenal cortex (Long, '47). Based on the finding that cholesterol enters into the formation of pregnandiol (Bloch, '45), it was suggested that cholesterol may be the precursor to cortical hormones. This suggestion is well supported by observations that the adrenal cortex suffers a depletion of its cholesterol under conditions requiring increased production of cortical hormones (Long, '47; Yoffey and Baxter, '49; Robinson and Yoffey, '50; Bergner and Deane, '48; Bacchus, '50a). Although changes of the ascorbic acid content of the cortex parallel those of cholesterol under various conditions (Long, '47), there is very scanty evidence to indicate the specific role of the vitamin in the cortex. Since the proposed association of the vitamin with certain cytoplasmic constituents (Bourne, '42) was shown to hold little or no significance, the only direct evidence that the vitamin is associated with cortical hormones comes from the isolation of a steroid-ascorbic acid complex from cortical tissue (Lowenstein and Zwemer, '46). There is indirect evidence to indicate that ascorbic acid, like the cortical hormones, is related to resistance (Perla and Marmorstan, '37).

This paper reports observations on the cytological distribution of cholesterol and of ascorbic acid in normal rats, and in rats exposed to the stress of cold (0–4°C.) for one hour, and for 48 hours.

MATERIALS AND METHODS

The work was conducted on 20 adult male Sprague-Dawley rats. Fourteen rats were placed, two in a cage, in a refrigeration room maintained at 0–4°C.; the animals were supplied ad libitum with Purina Laboratory Chow and tap water. Eight rats were sacrificed one hour, and 6 rats 48 hours, after introduction into the cold room. In addition 6 normal intact animals of the same colony as those above were sacrificed; these served as controls to the cold treated groups. Immediately after sacrifice the adrenals were excised and weighed to the nearest tenth of a milligram on a torsion balance. One adrenal from each animal was used for the detection of ascorbic acid, and the other for the detection of cholesterol.

The histochemical methods used were the same as those described in a previous paper (Bacchus, '50b). The method for detection of ascorbic acid employed the reducing property exhibited by ascorbic acid at a pH range of 2 to 2.5. Paraffin sections 6–7 μ thick were made of the silver treated tissues; some sections were cleared in xylene and mounted in Fisher's Permount and others were stained with Delafield's hematoxylin and eosin.

The gland used for the detection of cholesterol was initially fixed in 10% neutralized formalin prior to embedding in gelatin after the method of Zwemer ('33). Frozen sections 10–15 μ thick were made of the gelatin embedded tissue and they were treated the following ways: (1) stained with a saturated solution of Sudan IV in 70% ethanol, (2) for the Schultz reaction (Schultz, '24), (3) for the plasmal and the phenylhydrazine reactions (Dempsey and Wislocki, '46). Some sections were treated with acetone and then tested for the above reactions. Acetone soluble sudanophilic, Schultz-positive, substances were regarded as cholesterol or cholesterol derivatives, since the Schultz reaction probably detects the presence of diols formed from cholesterol after mild oxidation procedures (Everett, '47).

RESULTS

Weight data. The mean adrenal weight of the animals under stress for 48 hours is significantly higher than that of normal control animals ($P < 0.01$). The mean adrenal weight of rats under stress for one hour is higher than that of controls, but this is not statistically significant (table 1).

TABLE 1

Adrenal weights of rats under stress

STRESS	NO. RATS	FINAL BODY WEIGHT GM ± S. D.[1]	ADRENAL WEIGHT MGM ± S. D.[1]	P
(a) None	6	215 ± 5	35.9 ± 1.0	a–b $P > 0.05$
(b) 0°–4°C.: 1 hour	8	208 ± 8	37.3 ± 1.7	
(c) 0°–4°C.: 48 hours	6	205 ± 6	38.8 ± 1.2	a–c $P < 0.01$

[1] S. D. = $\sqrt{\frac{\text{Sigma } (x - X)^2}{n - 1}}$

X = Mean value

x = Individual value

Histological data. The distribution of cholesterol in the cells of the adrenal cortex is the same as described in a previous paper (Bacchus, '50b). In summary, cholesterol is present in the cells of the zona fasciculata and zona reticularis with a definite gradient of concentration, weakest in the reticularis and strongest in the fasciculata; a narrow sudanophobic zone, the transitional zone, lies between the zona fasciculata and the zona glomerulosa. The cells of the zone glomerulosa also contain large amounts of cholesterol. The distribution of sudanophilic lipids is co-extensive with that of cholesterol (fig. 1). The cholesterol was generally plasmal and phenylhydrazine positive, but in view of the questioned specificity of these two reactions (Feldman, '50; Danielli, '49; Boscott and Mandl, '49; Boscott, Mandl, Danielli and Shoppee, '48), emphasis is placed on the sudan and Schultz tests in this study. Reduced silver granules, indicating the presence of ascorbic acid, are present in all parenchymal cells of the adrenal cortex (fig. 2); in some cells the granules are evenly distributed in the cytoplasm, and in others, the granules are aggregated at the periphery of the cytoplasm (Bacchus, '50a, b).

A marked depletion of cholesterol from the cells of the zona fasciculata and the zona reticularis is observed in the adrenals of animals exposed to cold for one hour (fig. 3). Associated with this change is a profound depletion of ascorbic acid from these cells and the adjacent sinusoids. Whatever ascorbic acid is present in these cells is at the periphery of the cytoplasm (fig. 4). There is a marked reaccumulation of both these substances in the cells of the fasciculata and reticularis of animals exposed to the stress of cold for 48 hours (figs. 5 and 6). The ascorbic acid is present in large amounts in the inner sinusoids of the gland, but cells adjacent to these packed sinusoids are devoid of the vitamin.

DISCUSSION

It is observed that both cholesterol and ascorbic acid are discharged from the cells of the zona fasciculata and the zona reticularis of the cortex of animals exposed to sudden stress. This observation is well supported by chemical and cytochemical findings of several other workers. On the basis of the cholesterol pattern in these glands it is concluded that they are in the "alarm reaction" phase of the "general adaptation syndrome" (Selye, '50). While the depletion of cholesterol from the gland is explained by the release of cortical hormone, similar evidence for the discharge of ascorbic acid is lacking. Vogt ('48) was unable to find sufficient ascorbic acid (free or bound) in the adrenal effluent of dogs to account for the release of steroid-vitamin complexes as is suggested by the Lowenstein-Zwemer complex. The results of

Vogt's study indicated that the ascorbic acid contents of arterial plasma and of adrenal effluent plasma are at equilibrium. This observation alone, however, does not preclude the existence of steroid-ascorbic acid complexes since the study was conducted on dogs under anesthesia; the dogs were probably secreting hormones at a basal rate. The conclusion that, "the ascorbic acid content of the adrenal effluent cannot be used as an index of cortical secretion," is based on the predicted rate of discharge of ascorbic acid bound to a cortisone-like compound. In this connection it is well to note that in two adrenals otherwise showing signs of the alarm reaction, small amounts of ascorbic acid were observed in some cells and their adjacent sinusoids. This ocurrence may explain the "loss" of ascorbic acid which Vogt observed in the adrenal effluent of two dogs.

The distribution of the adrenal cholesterol after exposure to cold for 48 hours is indicative of the "resistance phase" of the "general adaptation syndrome." Robinson and Yoffey ('50) observed similar signs in rats exposed to cold for 72 hours; those workers did not study the distribution of ascorbic acid. It seems probable, therefore, that a reaccumulation of ascorbic acid in cells and sinusoids of the inner cortical zones might functionally be associated with the resistance phase.

The stress of cold did not appreciably affect the constituents of the cells of the zona glomerulosa. This observation confirms previous suggestions of the relative independence of the zona glomerulosa from the inner zones (Swann, '40; Deane, Shaw and Greep, '48; Bergner and Deane, '48; Bacchus, '50b).

It should be noted that the proposed association of ascorbic acid with certain cytoplasmic organs has not been ascertained in this study. It has been observed that the juxta-nuclear aggregation of ascorbic acid described by some workers is due to delay in the treatment of the tissue with silver nitrate (Deane and Morse, '48; Bacchus, '50b).

These findings suggest that ascorbic acid and cholesterol are involved in the secretory activity of the cortex. The specific role of ascorbic acid in the adrenal cortex is not known.

SUMMARY

A depletion of cytochemically detectable ascorbic acid from the adrenal cortex occurs in the alarm reaction. In the "resistance phase" of the "general adaptation syndrome" a reaccumulation of the vitamin is observed. These observations suggest that ascorbic acid is in some manner associated with cholesterol in the production of cortical hormones.

ACKOWLEDGMENT

The author acknowledges the aid of Doctor Warren Andrew and Doctor Chester E. Leese.

Doctor Raymund L. Zwemer rendered very helpful and constructive criticisms on methods.

Photomicrographs were made by Mr. Frank Delaney.

REFERENCES

Bacchus, H. 1950a Ascorbic acid and ketosteroid cytochemistry in the rat's adrenal cortex following prolonged injections of epinephrine. Fed. Proc., *9:* 7.

——— 1950b Cytochemical study of the adrenal cortex of the rat under salt stresses. Am. J. Physiol., *163:* 326–331.

Bergner, G. E., and H. W. Deane 1948 Effects of pituitary adrenocorticotrophic hormone on the intact rat, with special reference to cytochemical changes in the adrenal cortex. Endocrinology, *43:* 240–260.

Bloch, K. 1945 Biological conversion of cholesterol to pregnandiol. J. Biol. Chem., *157:* 661–666.

Boscott, R. J., and A. M. Mandl 1949 The histochemical distribution of ketosteroids in adrenal tissue. J. Endocrinology, *6:* 132–136.

Boscott, R. J., A. M. Mandl, J. F. Danielli and C. W. Shoppee 1948 Cytochemical demonstration of ketosteroids. Nature, *162:* 572.

Bourne, G. 1942 Cytology and Cellular Physiology. Oxford Univ. Press, 99–138.

Danielli, J. F. 1949 A critical study of techniques for the cytochemical demonstration of aldehydes. Quart. J. Micr. Sci., *90:* 67–74.

Deane, H. W., and Anna Morse 1948 Cytological distribution of ascorbic acid in the adrenal cortex of the rat under normal and experimental conditions. Anat. Rec., *100:* 127–142.

DEMPSEY, E. W., AND G. B. WISLOCKI 1946 Histochemical contributions to physiology. Physiol. Rev., *26:* 1–27.

EVERETT, J. W. 1947 Hormonal factors responsible for the deposition of cholesterol in the corpus luteum of the rat. Endocrinology, *41:* 364–377.

FELDMAN, J. D. 1950 Histochemical reactions of adrenal cortical cells. Anat. Rec., *107:* 347–350.

LONG, C. N. H. 1947 The relation of cholesterol and ascorbic acid to the secretion of the adrenal cortex. Recent Progr. Hormone Research, *1:* 99–122.

LOWENSTEIN, B. E., AND R. L. ZWEMER 1946 Isolation of a new hormone from the adrenal cortex. Endocrinology, *39:* 63–64.

PERLA, D., AND J. MARMORSTAN 1937 Role of vitamin C in resistance. Arch. Path., *23:* 543.

ROBINSON, F. B., AND J. M. YOFFEY 1950 Histochemical changes produced by cold and adrenaline in the suprarenal cortex of the adult male rat. J. Anat., *84:* 32–37.

SELYE, H. 1950 The Physiology and Pathology of Exposure to Stress. Acta, Inc., Montreal, Canada, pp. 286–346.

SCHULTZ, A. 1924 Eine Methode des mikrochemischen Cholesterinnachweiss am Gewebschnitt. Zentralbl. f. allg. Path. u. path. Anat., *35:* 314–317.

VOGT, M. 1948 Ascorbic acid in adrenal blood. J. Physiol., *107:* 239–243.

ZWEMER, R. L. 1933 Method for studying adrenal and other lipoids by modified gelatin embedding and mounting technique. Anat. Rec., *57:* 41–44.

PLATE 1

EXPLANATION OF FIGURES

1, 3, 5 are photomicrographs of Sudan-stained frozen sections of adrenals (10–15 μ).

2, 4, 6 are photomicrographs of 6 μ paraffin sections of adrenals treated with acid silver nitrate.

1 Distribution of sudanophilic lipids in normal adrenal. Note gradient of intensity. (Sudanophilic and Schultz-positive areas are co-extensive.) × 75.

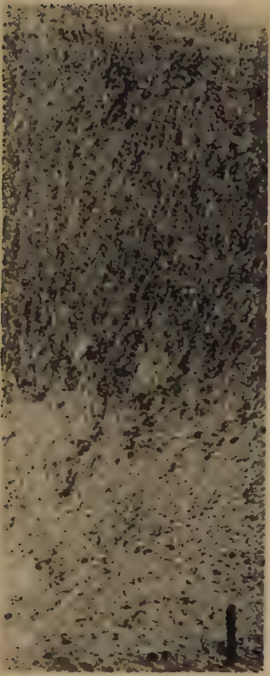
2 Hematoxylin counterstained adrenal of normal rat. Granules of reduced silver indicating ascorbic acid are present intra- and extra-cellularly. × 900.

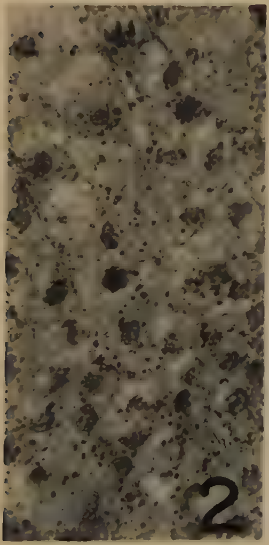
3 Distribution of sudanophilic lipids in rat submitted to 0–4°C. for one hour. Note depletion from zona fasciculata and zona reticularis (alarm ''reaction''). × 75.

4 Uncounterstained section of adrenal of rat submitted to 0–4°C. for one hour. Relative absence of ascorbic acid (''alarm reaction''). × 900.

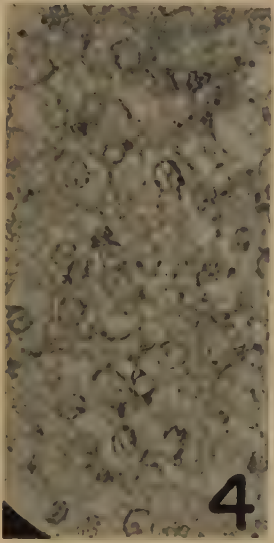
5 Distribution of sudanophilic lipids in rat submitted to 0–4°C. for 48 hours. Note reaccumulation of lipids in zona fasciculata and zone reticularis (''resistance phase''). × 75.

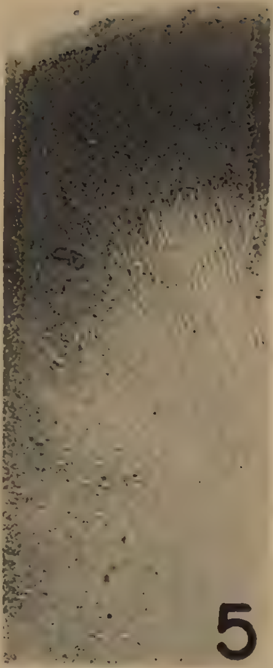
6 Uncounterstained section of adrenal of rat submitted to cold for 48 hours. Relative reaccumulation of vitamin in cells and in sinusoids. Relatively more vitamin is present in inner sinusoids (''resistance phase''). × 900.


1


2

3


4


5

6

ELECTRON MICROSCOPY OF NERVOUS TISSUE

DANIEL C. PEASE[1] AND RICHARD F. BAKER
Departments of Anatomy and Experimental Medicine, The Medical School,
University of Southern California, Los Angeles, California

The present study of nervous tissue with the electron microscope is frankly exploratory in character. The development of a suitable technique of ultrathin sectioning necessarily had to precede the effective use of the electron microscope for such a problem. This has been accomplished to the extent that 0.1–0.05 μ sections were obtained routinely. The details of the method have been published elsewhere (Pease and Baker, '48, '50, and '51).

This report concerns itself in part with a study of spinal cord, and in part with peripheral nerve. Work on the former was completed over a year before the latter was begun. Certain technical improvements were made during this interval so that the two studies are not entirely comparable.

Most of our work has been with magnifications between 6,500 and 17,500 diameters. Of course these are low magnifications for the electron microscope, but observations could be correlated to a large extent with knowledge derived from light microscopy. Also, at such magnifications, we have not been handicapped seriously by the limitations of conventional fixation. The resolving power, however, was sufficient to reveal some hitherto obscure details with great clarity.

Since our findings concern diverse subjects, it will be convenient to combine observations and conclusions under several topics.

MATERIAL AND METHODS

The entire study has been made on nervous tissue of young adult rats. The animals were prepared by an intraperitoneal amytal anaesthetic. Spinal cord material was obtained by perfusing the left ventricle of the heart with fixative immediately following general perfusion with buffered Locke solution. It took about 5 minutes after the thorax was first opened to complete this procedure. Peripheral nerve was obtained by the direct application of fixative as described below.

---

[1] Present address, Anatomy Department, the Medical School, University of California at Los Angeles, Los Angeles, California.

The most valuable fixative proved to be 2% osmic acid. Not only did it preserve the myelin but, in the words of Heidenhain ('11), "weiterhin ist bekanntlich die Osmiumsäure das einzige Mittel, um die Achsenfaser in voller Breite, ungeschrumpft, zu erhalten, während alle übrigen Fixierungsmittel . . . zu enormen Schrumpfungen der Fasser führen" (p. 883). Zenker's and Flemming's fluids were useful for Nissl bodies, and the former occasionally fixed axones without shrinkage. Ten per cent formol was employed also, but produced such coarse fixation that it was abandoned quickly. Perfused carcasses were kept about 16 hours before the tissue was removed, and dehydration begun.

The blackening effect of osmic acid was a good gross indication of adequate fixation. Upper cervical cord was found to be well fixed most consistently. No doubt this was because of the special circulation to this region by way of the vertebral arteries. We confined our studies to this region. Furthermore, it was quite obvious, both macroscopically and microscopically, that grey regions tended to receive more fixative than the white columns. The well fixed regions, therefore, corresponded to the distribution of the anterior spinal artery rather than the posterior. As a consequence, the best preserved white matter usually was found in reticular areas.

Peripheral nerve was obtained from the axilla. The gross fascia was removed from the nerves of narcotized rats, but otherwise the region was disturbed as little as possible. The walls of the fossa served to make a cup into which 2% osmic acid was dropped continuously for about 15 minutes. At that time the nerves were thought to be partly fixed, and were cut and removed from the still living rat. Osmic acid was the only fixative used with peripheral nerve, and it was allowed to act 16–24 hours. It was washed out in several changes of dilute alcohol.

The references cited in the introduction explain the embedding, sectioning and mounting techniques which were employed.

RESULTS AND CONCLUSIONS

The cell surface. Figure 1 is of a reticular area of spinal cord. In the lower left, particularly, large numbers of axones have been sectioned transversely. Their dense myelin sheaths appear as black rings. The axoplasm is pale. The body of a large nerve cell, probably a ventral horn motor cell, lies in the upper right quadrant. A large dendritic trunk extends down towards the bottom of the figure. The plane of section almost missed the nucleus. Figure 2 is of a much smaller nerve cell, but illustrates the nucleus very well.

In many places it is hard to distinguish the outline of these nerve cells with precision. This leads to the first conclusion, that a striking characteristic of the cell surface is an extraordinary thinness of the limiting membrane. It is an altogether finer structure than the nuclear membrane (figs. 2 and 3). It is so thin that it cannot be seen in most places when closely applied to adjacent structures. Occasionally, however, it stands out from surrounding material as a very fine line, essentially as thin as the finest threads of the reticulated cytoplasm. The arrows indicate where it may be seen to advantage in figures 1, 2, and 3.

Undoubtedly the observable membrane is deceptively thickened in many places as a result of a diffuse precipitation upon it. This was particularly true when fixatives other than osmic acid were employed, but at favorable loci, the limiting membrane was distinguished from any precipitate by its uniform sharpness and its intrinsic density.

We have not worked with sufficiently great magnifications to measure the thickness of the surface membrane accurately. But at most it is a few hundreths of a micron thick. The nuclear membrane, on the other hand, is close to 0.1 μ in thickness. In all probability, therefore, the surface of the nerve cell consists of a plasma membrane alone, having the thickness of only a few molecular lengths.

It is to be emphasized that there is no evidence of any plasmagel layer. If the latter were present, we should expect to see it as a region of increased density underlying the surface membrane. Thus there is no specialized internal structure visible to support the cell surface.

It is not only the cell body which possesses such a thin delimiting membrane. Axones, too, have the same sort of plasma membrane at their surface. Ordinarily this cannot be seen, for it is applied directly to the myelin sheath in well fixed regions. However, occasionally axones have been found mechanically torn away from the myelin during sectioning. Under these circumstances, the axone surface has been exposed, and can be seen as a fine line, as indicated by the arrows in figure 5.

The cytoplasm. Electron micrographs provide strong evidence that nerve cells have a relatively high water content, for they give semi-quantitative information about the amount of material present. This is because the electron image fundamentally reflects mass-density differences, somewhat as does an x-ray picture.

It can be seen in figures 1 and 2 that there is only a modest amount of precipitated protein present in cytoplasmic areas. Of course this is observed in fixed and dehydrated cells, but it can be presumed that most of the protein has been retained. The cytoplasmic picture resembles that of comparably prepared liver (Pease and Baker, '48), or of the collecting tubules or Henle loop cells of the kidney (Pease and Baker, '50). It supports the contention of Hoerr ('36) that the protoplasm of nerve cells cannot be stiffer than that of other "watery" cells.

The axoplasm apparently is slightly more dilute even than the cytoplasm elsewhere. The sparse precipiate is obvious in figures 4, 5, and 10. In this respect, figure 1 is particularly revealing in that axoplasm can be compared directly with cytoplasm in a single section. With poor fixation, the whole picture changes. Then shrivelled axones are found with only a fraction of the cross-sectional area of properly fixed ones. Under such circumstances, the axoplasm often looks uniformly dense.

At this point it may be well to comment further upon the influence of fixation. Figures 1 and 2 are to be compared with figure 3. The latter is of a cell fixed with Zenker's fluid. The cytoplasm and nucleoplasm appears rather coarsely precipitated. The contrast with osmic acid fixation is even greater if white matter is compared. The cell of figure 3 is surrounded by axones, but these are so shrivelled and distorted that they are difficult to pick out.

Neurofibrils as artefacts. No one with experience in cytological technique doubts but that coarse fibrous structures can be precipitated by poor fixation. For this reason, there has been a group of neurocytologists who always have regarded neurofibrils as artefacts. Certainly very often, at least, their appearance is coupled with an obvious shrinkage. However, the matter remains a controversial subject. We feel obliged to enter the discussion since we have seen nothing identifiable as a neurofibril except in obviously shrunken and poorly fixed material, and except in the special position of nodes of Ranvier in peripheral nerve.

In doubting the existence of neurofibrils in the conventional sense, we do not question the probability of some linear organization of colloids. In living giant axones of squid, birefringence studies of Bear, Schmitt, and Young ('37) have shown a longitudinal organization of micellar particles. In well fixed axones, what are probably traces of this sometimes remain in that the feltwork of precipitated protein often shows a predominantly longitudinal organization. We have not deemed it worthwhile to select a micrograph to show this clearly, but it does show to some extent even in the large dendrite of figure 1. At most, poorly defined fibrils of low density are seen, that would be altogether too fine to be visible with the light microscope. Thus we believe that there is an oriented colloidal organization in axoplasm (and in dendrites), but that the protoplasm is too greatly hydrated to be called fibrous in any ordinary sense of the word. Fixatives which produce shrinkage (dehydration) may precipitate fibers.

We have commented already that nodes of Ranvier are exceptional in that fibrils are definitely to be seen in these loci even in well preserved material. They can be seen fairly clearly in the upper portion of figure 12 *(n.f.)* although this figure was not selected for this purpose. They also show in figure 11. The fibrils extend but a short distance beyond the nodes. The presence of fibrils here may be correlated with an increased axoplasmic density in the nodal regions.

In considering the controversy about neurofibrils, one further comment seems worthwhile. It is widely quoted that fibrils have been seen in living tissue culture cells, and this is taken as proof of their normal existence. However, Weiss and Wang ('36) who made the original observation, were by no means certain that this was so. They pointed out that in any case the fibrillar structures visible to them were altogether coarser fibers than those found in silver preparations.

Nissl bodies as precipitates. In talking of Nissl granules we shall be forced to continue the discussion of fixation artefacts, for they have been visible to us only after what we should call coarse fixation. They were identifiable after Zenker, Flemming, or formol treatment (fig. 3, particularly the insert), but they were not to be seen after good osmic acid fixation (fig. 1). On the other hand, they did appear when osmic acid fixation had not been optimal, so that the ground substance was somewhat coarsely reticulated (small Nissl granules probably show in fig. 2).

The literature abounds with discussion as to whether or not Nissl bodies are carefully cultivated fixation artefacts. As indicated above, our own limited observations lead us to believe that they probably are, for we are now in a position to characterize the ground substance after fixation, and make a correlation with the presence or absence of Nissl bodies.

However, we do not have enough comparative data for a definite proof. The best evidence for the contrary view, that they exist in life, is open to some question. They have been photographed with ultraviolet light in "living" cells, but, of course, only after teasing the cells in saline (Wiemann, '25). The tissue culture micromanipulative evidence of Levi and Meyer ('45) is also open to much the same doubt. They have been seen in lyophilized material which admittedly contained ice crystal artefacts, and subsequently was exposed to aqueous stains (Bensley and Gersh, '33). The work of Beams and King ('35), however, does indicate Nissl material to be present at least as a colloid, for it can be displaced with centrifugal force before fixation.

When Nissl bodies have been seen with the electron microscope, they appeared as coarse, irregular aggregates. Within the limits of our resolution, they did not seem to be made up of unit particles of any constant size. Rather, one might characterize them as having the appearance of a flocculent precipitate without definable order or, sometimes, as diffusely non-granular masses. They usually looked like the chromatin clumps within adjacent nuclei.

Mitochondria. Mitochondria were obvious bodies in nerve cells after fixation with osmic acid. They appeared as dense masses widely scattered. They tended to be spherical in the cell bodies, and elongated in dendrites (fig. 1). They were small, measuring about a quarter or a third of a micron in diameter. Because of their size, they were most easily seen when fixation produced a finely reticulated background. After poor fixation they tended to be buried in the coarse precipitate, and were hard to find. Even, then, however, enough mitochondria could be seen to demonstrate their presence. Thus there is no suggestion of their being fixation artefacts.

In axones, mitochondria were seen to be somewhat specialized. They occurred as long filaments. The length apparently was indefinite, but the diameter was quite regular (fig. 4). In transverse sections of axones they appeared as dots (fig. 5).

The number of "dots" in any particular cross section of an axone varied from 0 to at least 6. Thus the mitochondrial filaments tended to overlap each other in an irregular manner.

In other tissues, particularly in parts of the kidney, it has been possible to visualize internal detail in mitochondria (Pease and Baker, '50). In all of our nervous tissue, however, mitochondria have looked essentially homogeneous. In part, this may depend upon the size differences, but it may also reflect differences in physiological activity.

The Golgi apparatus. The Golgi apparatus should be mentioned in passing in this acount of cell inclusions. We have not seen it at all, presumably because of the fixation methods that we employed. This will surprise no one familiar with the problem.

The medullary sheath. From our own observations it would seem that every axone in the spinal cord has a medullary sheath by the time it leaves grey matter. It is interesting that many medullated axones can be seen with diameters (including the sheaths) substantially less than 1 μ. In fact, occasional medullated axones less than 0.5 μ in diameter have been seen. It is not hard to observe this in the white matter of figures 1 or 5. Because of their small size (and in the absence of any other recognizable type), we believe that such small axones correspond to those generally called "non-myelinated." Dense sheaths they have without a doubt, but the density does not necessarily indicate stainable myelin!

In peripheral nerve, the medullated fibers can be seen without difficulty (fig. 10) and truly unmedullated fibers may be present also. We are not sure that we recognize them, but if one looks at the upper part of figure 10 there is a rather amorphous mass, partly compartmentalized, and having the general appearance of axoplasm. There are even suggestions of mitochondria. Such masses are characteristic of peripheral nerve. They may represent connective tissue elements. We think, however, that they resemble the syncytial masses of Schwann cells containing unmedullated fibers as described by Nageotte ('32). Further study will be necessary to clarify this point.

The myelin sheaths of large peripheral nerve fibers can be substantially thicker than those of the cord on fibers of comparable size (figs. 1 and 5 compared with fig. 10). In peripheral nerve in particular we have noted that the myelin may, on occasion, look vacuolated in such a way that the vacuoles form a fairly regular grid. At other times extremely dense inclusions have been seen scattered as small dots throughout an otherwise homogeneous myelin. These patterns may reflect aspects of the structural arrangement of myelin, but without further information, we cannot draw useful conclusions, and so do not even illustrate the findings. Apparently Rozsa, Morgan, Szent-Györgyi and Wyckoff ('50) have seen some of these patterns, and have been puzzled also about how they should be interpreted.

At nodes of Ranvier, however, the myelin is sometimes arranged in such a way as to indicate that it is laminated. Figures 11 and 12 have been chosen particularly to illustrate this point. As the heavy myelin sheet approaches the node, definite layers apparently have peeled off successively. The main sheet tapers as the different lamellae leave it. Thus at least 6 layers can be distinguished in some micrographs.

The neurilemma. The neurilemmal sheath is not conspicuous in most micrographs of peripheral nerve. This is because it is but a little less dense than myelin, and is applied directly to the myelin. When there has been shrinkage, however, as in the section of figure 10, the myelin may pull away from the neurilemma so that the latter appears as an obvious sheath on most neurons. In optimally fixed neurons, as in figure 9, the separation does not exist.

There are conflicting views in the literature about the character of the neurilemma and its relation to Schwann cells. In many places Schwann cell nuclei have been seen interposed between the neurilemma and the myelin. One rather poor example appears in figure 10 *(Swn.)*. It has not been possible, however, to define the cytoplasm of Schwann cells except in the immediate vicinity of nuclei. If it exists as a sheath over the whole nerve fiber, it is too attenuated to be distinguished from the neurilemma. The neurilemma, on the other hand, is at least partly made up of collagenous fibrils and as such is almost certainly an extracellular layer.

Figure 8 proves the collagenous character of the neurilemmal fibrils. Short lengths of fine fibrils were scattered by a dull knife in the vicinity of an axone. These fibrils are transversely banded with an axial periodicity of 640 Å. This corresponds to the periodicity of collagen as determined by Schmitt, Hall, and Jakus ('42). It also corresponds to the periodicity of what de Robertis and Schmitt ('48) have called "neurotubules." The latter were fibrous structures extracted from peripheral nerve, and originally were thought to come from within axoplasm.

In figure 8, the relationship between the fibrils and the nerve is not apparent. The fibrils had been smeared out by poor sectioning, and apparently it is because of this isolation that the banded structure can be seen so clearly. The axone in the small insert from the same material has a neurilemma that is still recognizable. The contained fibrils were smeared enough, however, so that their banded structure shows upon enlargement. Thus there is no doubt that these fibrils belong to the neurilemma, and are the same as those which show in normal relation to the nerve in figure 9.

Figure 9 requires a little study for orientation. Peripheral nerve fibers have been sectioned longitudinally. In some regions axoplasm *(ax'pl.)* is visible. The very dark mottling, however, is where sheets of myelin *(myl.)* lay in the horizontal plane. Then, in other places, the neurilemma *(n'lem.)* is viewed horizontally, showing fibers which course longitudinally. The principal strip in figure 9 *b* thus is a surface view of neurilemma.

Figure 9 makes it obvious that the principal fibers of the neurilemma run longitudinally. It is not altogether clear to us how they are held in this position. In a few micrographs suggestions of a transverse system of small fibers have appeared. Thus there may be a fibrous lattice. This will have to await further clarification.

The synaptic junction, glial relationships, and other structural aspects of grey matter. There remain to be considered a variety of things that occur within grey matter. It must be admitted at the outset, however, that many features of grey matter have defied analysis so far. Thus grey matter contains at least three types of fine fibrous structures which have not yet been differentiated fully. This list includes terminal dendritic branches, telodendria with their end feet near the synaptic junction, and glial fibers.

Consider first the synaptic junction. By any statistical calculation we must have seen hundreds of end feet on nerve cell bodies and dendrites. Yet we have not recognized even one with certainty. It is true that probably some are to be seen in the upper right of the cell of figure 1, but the telodendria, if such they are, cannot be traced far enough to be really convincing. The ultrathin sections necessary for electron microscopy preclude following such structures appreciable distances unless they happen to lie in the plane of the cut. Serial sections may obviate this limitation eventually, but difficulties with mounting make them impractical at the present time. What appear to be end feet in figure 1 really might be oblique sections of glial fibers, or, perhaps, terminal dendritic arborizations from neighboring cells. The task of differentiating is by no means hopeless, of course, for fortunate sections, or different fixation might provide clues at any time. The main difficulty of the moment is, thus, in the recognition of what actually shows in the electron micrographs before one's eyes.

Figure 6 shows a typical section of grey matter except that no cell body was in the field selected. The complexity of detail is bewildering. Figure 7 looks quite different, consisting of much more minute structural parts. It is thought that figure 7 is from the substantia gelatinosa, while figure 6 represents spongiosa. It is interesting that in figure 7 multitudes of tiny processes can be seen with clarity and measured accurately. In transverse sections these appear to have the essential structure of axones, with a pale core, but dense sheath. Thus they look like tiny doughnuts. They grade down in size at least to a quarter of a micron. It is likely that they represent telodendria. The large numbers present are consistent with Cajal's famous figure of axone terminations in the cord which shows axones arborizing more elaborately in the gelatinosa than in any other place.

Finally, it may be admitted that we have not searched seriously for glial cell types. The two small cells visible in figure 7 might be glial, or nervous. It will take much more work to solve all of the riddles of grey matter. Our efforts so far should be regarded as being essentially a survey rather than an exhaustive study.

DISCUSSION

Since preparing this manuscript, three reports having important bearing upon this work have come to the authors' attention. Fernandez-Moran ('50) employed a variety of preparation techniques including sectioning to study the sheaths of peripheral nerve fibers. He has obtained a great deal of interesting information on the internal organization of the myelin sheath. In a variety of ways, including after alcohol extraction, and tryptic digestion, he has demonstrated a lamellar structure in the fixed nerve. Fernandez-Moran also has good micrographs showing that the neurilemmal sheath consists principally of collagenous fibers running longitudinally.

Schmitt and Geren ('50) have reinvestigated the question of "neurotubules" as described originally by de Robertis and Schmitt ('48). They have obtained sections which indicate the collagenous character of the neurilemmal sheath. They recognize the fact that these correspond to the neurotubules. Thus there is agreement about this important question.

Micrographs of Rozsa, Morgan, Szent-Györgyi and Wyckoff ('50) show most clearly that there is no axilemma.

SUMMARY

Ultrathin sections of rat spinal cord and peripheral nerve have been examined with the electron microscope. Several fixatives were employed by a perfusion method, and, of these, osmic acid proved best in avoiding shrinkage and in producing the most delicate coagulum.

The nerve cell surface was bounded by an extremely thin membrane only a few hundred angstrom units thick at most. It was substantially thinner than the nuclear membrane. It is reasonable to believe that it was only a plasma membrane of molecular dimensions.

Electron micrographs reflect density differences. Thus it is possible to say that nerve cells have a relatively watery cytoplasm. Axoplasm has an even higher water content than the cell body.

Neurofibrils in the conventional sense are regarded as artefacts of fixation. When fixation is optimal, coarse fibrillar structures are to be seen only in the immediate vicinity of nodes of Ranvier in peripheral axones.

Nissl bodies appeared as large masses when fixation was coarse. It is thought that these represented a flocculent precipitate. The idea that Nissl material is present normally in colloid form is not questioned.

Mitochondria are described. The Golgi apparatus was not seen, presumably because of the fixation employed.

Medullary sheaths were present on every axone of the white matter of the cord. This included axones only 0.5 μ in diameter. Truly unmedullated axones may have been seen in peripheral nerve.

There was evidence that myelin exists in the form of laminated sheaths which tended to separate at nodes of Ranvier.

The neurilemmal sheath of peripheral nerve was found to contain longitudinal fibers having the periodically banded structure of collagen. A transverse system of fibers may exist also. Schwann cells underlie the neurilemma but their cytoplasm proved to be too attenuated to define.

A number of features of grey matter remain to be clarified. For example, telodendria, terminal dendritic branches, and glial fibers all are minutely thin structures that cannot be differentiated easily. However, it is likely that telodendria have been identified in the substantia gelatinosa of the cord. There they have dense sheaths (or walls) and a watery core. Their diameters may be as small as 0.25 μ.

LITERATURE CITED

BEAMS, H. W., AND R. L. KING 1935 The effect of ultracentrifuging the spinal ganglia cells of the rat, with special reference to Nissl bodies. J. Comp. Neur., *61:* 175–184.

BEAR, R. S., F. O. SCHMITT AND J. Z. YOUNG 1937 The ultrastructure of nerve axoplasm. Proc. Roy. Soc. Lond., B, *123:* 505–519.

BENSLEY, R. R., AND I. GERSH 1933 Studies on cell structure by freezing-drying method. III. The distribution in cells of the basophil substances, in particular the Nissl substance of the nerve cell. Anat. Rec., *57:* 369–385.

FERNÁNDEZ-MORAN, H. 1950 Sheath and axon structures in the internode portion of vertebrate myelinated nerve fibers. Exp. Cell. Res., *1:* 309–340.

HEIDENHAIN, M. 1911 Plasma und Zelle. In Handbuch der Anatomie des Menschen, *8,* pt. 2, (ed. K. von Bardeleben), Gustav Fischer, Jena.

HOERR, N. L. 1936 Cytological studies by the Altmann-Gersh freezing-drying method. III. The pre-existence of neurofibrillae and their disposition in the nerve fiber. Anat. Rec., *66:* 81–90.

LEVI, G., AND H. MEYER 1945 Reactive, regressive, and regenerative processes of neurons cultivated in vitro and injured with micromanipulation. J. Exp. Zool., *99:* 141–181.

NAGEOTTE, J. 1932 Sheaths of the peripheral nerves. Nerve degeneration and regeneration. Section 5 in Cytology and Cellular Pathology of the Nervous System, *1* (ed. W. Penfield), Paul B. Hoeber Inc., New York.

PEASE, D. C., AND R. F. BAKER 1948 Sectioning techniques for electron microscopy using a conventional microtome. Proc. Soc. Exp. Biol. and Med., *67:* 470–474.

——— 1950 Electron microscopy of the kidney. Am. J. Anat., *87:* 349–389.

——— 1951 Triple embedding for the ultrathin sectioning required by electron microscopy. Anat. Rec., *110:* 531–538.

DE ROBERTIS, E., AND F. O. SCHMITT 1948 An electron microscope analysis of certain nerve axon constituents. J. Cell. and Comp. Physiol., *31:* 1–23.

ROZSA, J., C. MORGAN, A. SZENT-GYÖRGYI AND R. WYCKOFF 1950 The electron microscopy of sectioned nerve. Science, *112:* 42–43.

SCHMITT, F. O., AND B. B. GEREN 1950 The fibrous structure of the nerve axon in relation to the localization of "neurotubules." J. Exp. Med., *91:* 499–504.

SCHMITT, F. O., C. E. HALL AND M. A. JAKUS 1942 Electron microscope investigations of the structure of collagen. J. Cell. and Comp. Physiol., *20:* 11–33.

WEISS, P., AND H. WANG 1936 Neurofibrils in living ganglion cells of the chick, cultivated in vitro. Anat. Rec., *67:* 105–117.

WIEMANN, W. 1925 Studien am Zentralnervensystem des Menschen mit der Mikrophotographie im ultravioletten Licht. Zeitschr. f. d. ges. Neur. u. Psychiat., *98:* 347–404.

PLATE 1

EXPLANATION OF FIGURE

1 A reticular area of cord. The body of a large nerve cell fills the upper right quadrant. One of its dendritic trunks extends down to the lower margin of the figure. The plane of section almost missed the nucleus *(nuc.)*. Small arrows point out where the cell margin can be seen to advantage. Small, spherical mitochondria *(mit.)* are present.

The lower left quadrant of the figure is occupied by a bundle of myelinated axones sectioned transversely. The myelin sheaths appear as black circles. In the center is the pale axoplasm. The 10 μ scale at the top of the figure includes a 1 and a 0.5 μ circle which can be used to estimate the size of particular axones. Some have diameters of only 0.5 μ. × 4,500.

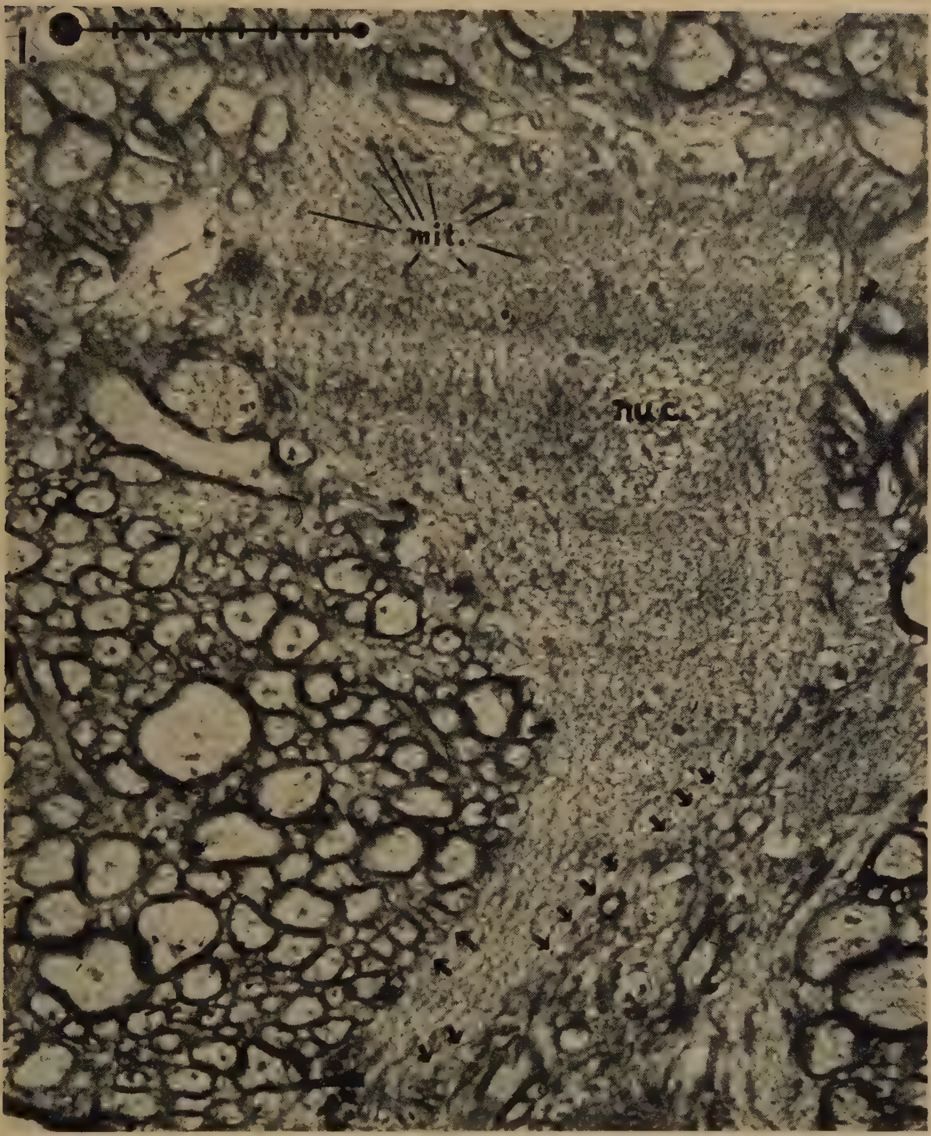

1.
mit.
nuc.

PLATE 2

EXPLANATION OF FIGURES

2 and 3 are of moderate sized nerve cells. The nuclear membrane shows well in both. Note that it is substantially thicker than the cell membrane. Arrows indicate where the latter can be seen to advantage. × 4,500.

3 differs from all the others in this report in that Zenker's fixative was used instead of 2% osmic acid. The fixation was more coarse. This is particularly obvious in the white matter, and in the aggregation of chromatin clumps in the nucleus. It seems to be under these circumstances that Nissl bodies *(Nissl.)* appear. They show especially well in the insert. They are similar in appearance to chromatin clumps in the nucleus. × 4,500.

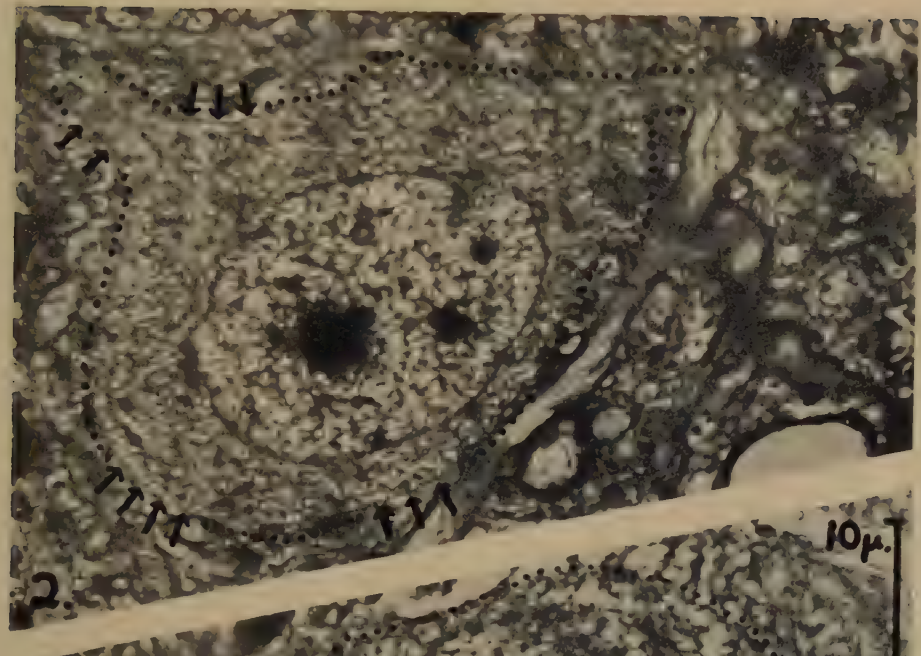

2.
3.
10µ.
Nissl
Nissl

PLATE 3

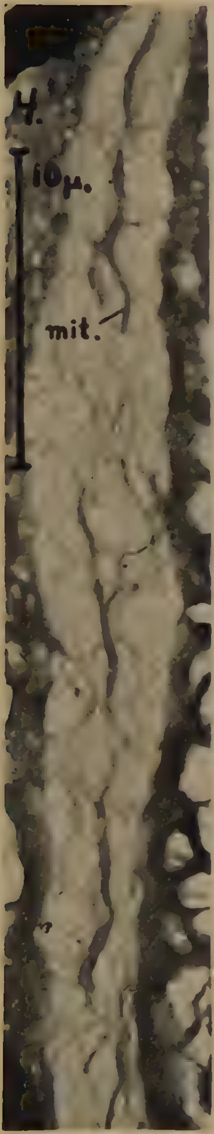
EXPLANATION OF FIGURES

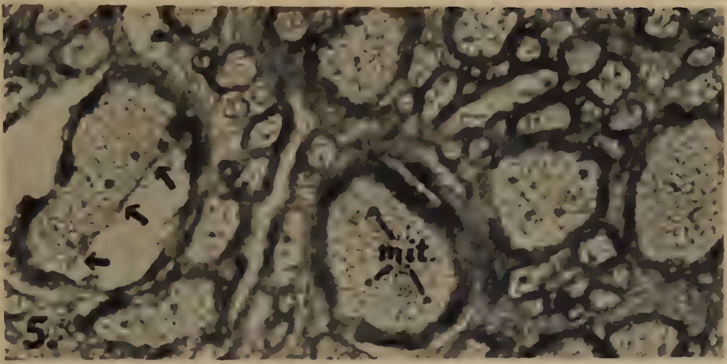
4 A longitudinal section of a large axone. Filamentous mitochondria *(mit.)* can be seen in the axoplasm. × 4,500.

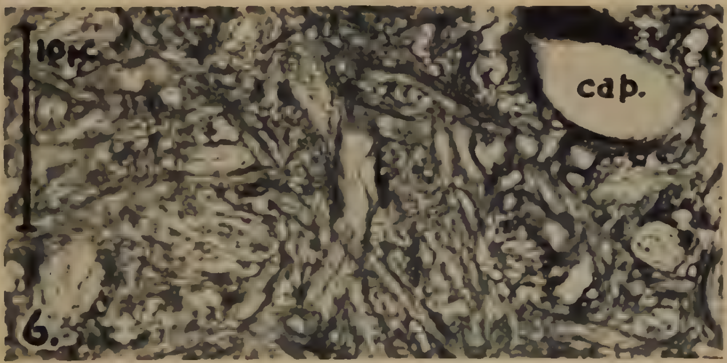
5 Transverse sections of axones at the same magnification as figure 4. Mitochondria appear as dots. The arrows point to a locus where the axone membrane was pulled away from the myelin. Note that in spite of large variations in axon diameter, the thickness of myelin sheaths shows but little difference. × 4,500.

6 Typical grey matter selected to avoid cell bodies. Medullated axons can be recognized without difficulty. Pale areas, without a definite sheath, represent dendritic trunks. Thus the figure number lies on the border of a dendrite sectioned transversely. A capillary or small vein *(cap.)* is indicated. × 2,800.

7 Grey matter thought to come from the substantia gelatinosa at the same magnification as figure 6. Two small neurons or glial cells can be found lying just above the single longitudinal section of a large axone at the lower left of the figure. It is only the cell nuclei which are conspicuous. The tiny dense rings with pale centers are thought to be transverse sections of telodendria. Some are but $0.25\,\mu$ in diameter. × 2,800.


4.
10µ.
mit.


mit.
5.


10µ.
cap.
6.

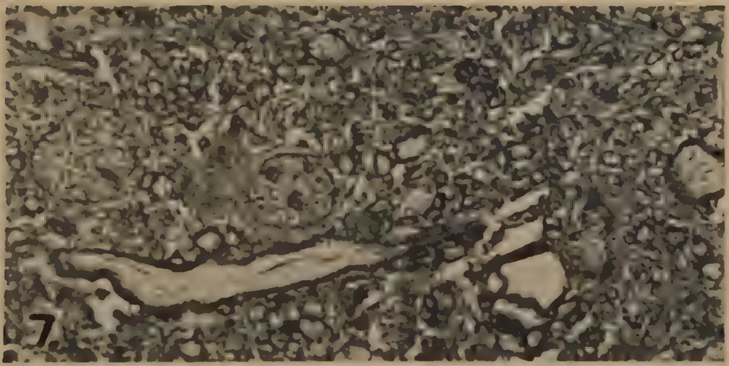
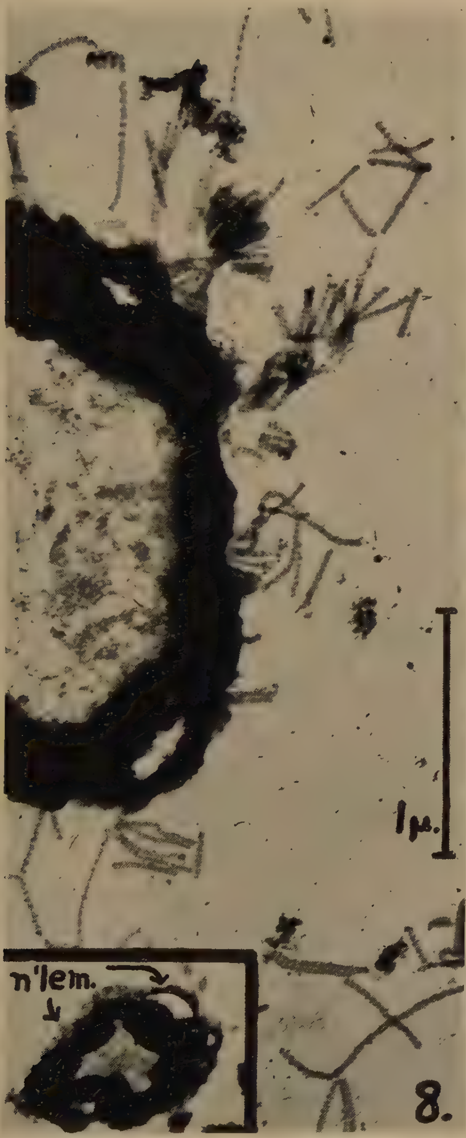

7.

PLATE 4

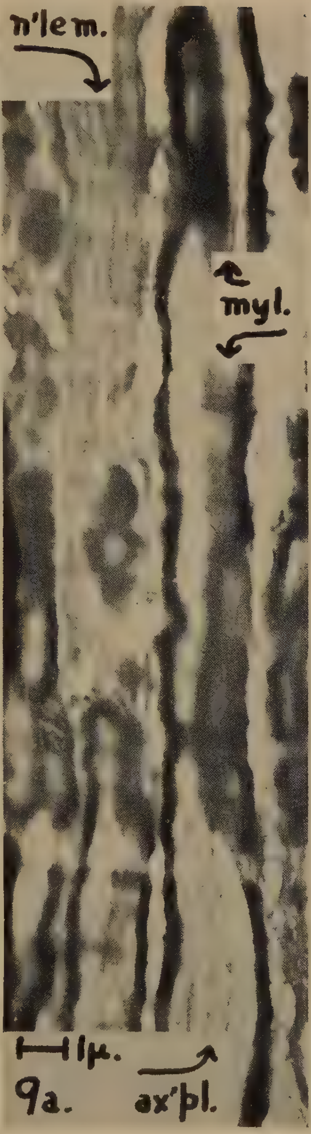
EXPLANATION OF FIGURES

8 A rather thick section of an axone of peripheral nerve shows to the left of the figure. Short lengths of fibers derived from the neurilemma are scattered in the vicinity. The fibrils show a banded structure with an axial periodicity of 640 Å. This corresponds to the periodicity of collagen. × 33,000. The small insert in the lower left corner is from identical material, but the neurilemma was only partly scattered. Thus the relationship of the fibrils can be established.

9 Longitudinal sections of peripheral axones. In places the axone cylinders have been sectioned and axoplasm is indicated *(ax'pl.)*. In other loci the dense myelin sheaths *(myl.)* lie in the horizontal plane and are seen as darkly mottled areas. Most important, the neurilemma *(n'lem.)* can be viewed horizontally also, and shows a longitudinally oriented fiber structure. This is the principal component visible in figure 9 b. × 6,000.



8.



9a.

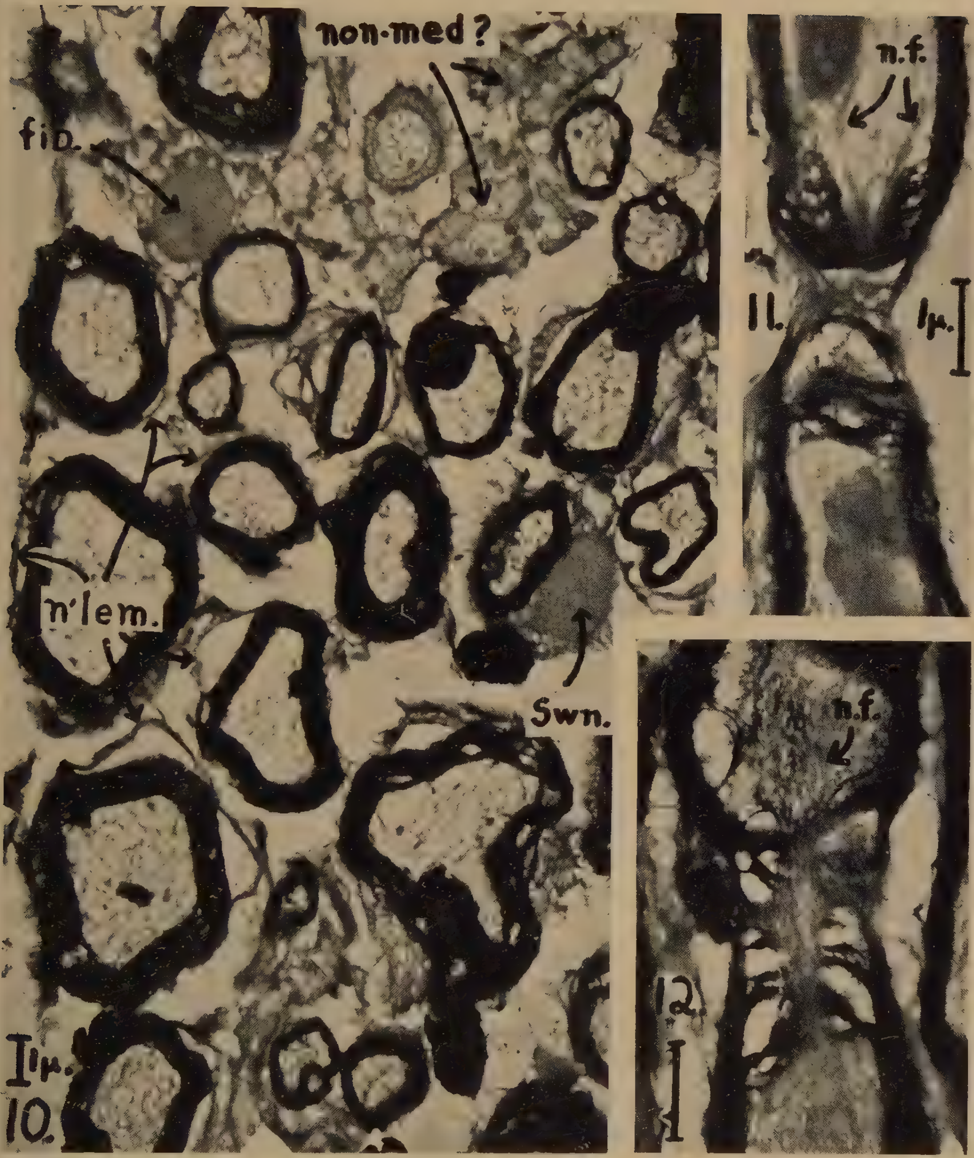
9b.

PLATE 5

EXPLANATION OF FIGURES

10 Transverse section of peripheral nerve. Fixation produced some shrinkage so that axons pulled away from their neurilemmal sheaths *(n'lem.)*. The nucleus of one Schwann cell *(Swn.)* shows, underlying the neurilemma. An endoneurial fibroblast *(fib.)* also is indicated lying outside of neurilemma. There may be non-medullated axones *(non-med?)* in the tissue mass in the upper part of the figure. × 6,000.

11 and 12 Two longitudinal views of nodes of Ranvier from peripheral nerve at a higher magnification than figure 10. Of particular interest is the way myelin has peeled off in sheets at the node suggesting a laminated structure. At the top of figures 11 and 12 fibrous elements *(n.f.)* show to the advantage in the axoplasm. These are the only structures comparable to ''neurofibrils'' which can be found, and have only been seen in the immediate vicinity of nodes. × 12,250.


non-med?
fib.
n'lem.
Swn.
1µ.
10.
n.f.
11.
1µ.
n.f.
12

TRIPLE EMBEDDING FOR THE ULTRATHIN SECTIONING REQUIRED BY ELECTRON MICROSCOPY[1]

DANIEL C. PEASE[2]

Departments of Anatomy and Experimental Medicine, The Medical School, University of Southern California, Los Angeles, California

THREE FIGURES

Electron microscopy is intrinsically limited to very thin specimens. Ultrathin sectioning therefore is the only general method adaptable to histological problems. Since good sections generally must not be more than 0.1 μ in thickness, there is a considerable problem in obtaining them.

Very high speed microtomy developed by Fullam and Gessler ('46) produced a few good sections, but proved costly and inefficient. Pease and Baker ('48) introduced the first effective technique that adapted a standard microtome. It depended upon simple modifications and refinements of standard procedures. The latters' original method was improved as time went on, both by these and other investigators. The paper of Pease and Baker ('50) describes the technique as it was employed in this laboratory immediately before the improvement described in the present paper.

Ultrathin sectioning depends upon giving adequate support to the specimen. Rigidity is needed in the block to be cut, and elastic strength in the section after cutting. Thus ordinary paraffin embedding is useless because the wax crushes. Our own basic method employed double embedding.

---

[1] This work was aided by a grant from the National Cancer Institute, of the National Institute of Health, the Public Health Service.

[2] Present address, Anatomy Department, The Medical School, University of California at Los Angeles, Los Angeles, California.

The tissue was impregnated first with low viscosity nitrocellulose almost saturated in equal parts of ether and absolute alcohol (approx. 40%). The block then was hardened in chloroform, and impregnated with a mixture of 3 or 4 parts of hard paraffin (m. p. 65°C.) and one part of bayberry wax. A block prepared in this way is fairly hard, and sections once obtained are reasonably tough. After practice, soft tissues usually can be sectioned fairly easily and consistently provided mechanical precautions are followed, and the knife is sharp. But we must admit that the technique is exacting and without much latitude. With tissue containing a great deal of fiber (as dermis), the support has proved just barely adequate. With hard tissue, such as hair, Baker and Pease ('49) were forced to use sub-zero temperatures to achieve the further hardening which proved necessary.

It has occurred to us that there was opportunity to add an additional substance to harden the block further, and that this might effect a great improvement. What was required was a substance soluble in chloroform, yet insoluble in paraffin. This could be infiltrated into the block after the nitrocellulose impregnation. Then, after soaking in chloroform, the solvent could be removed by molten paraffin, leaving the solute behind. With this thought in mind, we have tested several resins and one plastic with very gratifying results. There are large numbers of potentially useful compounds that might be employed.

In all cases we have made very viscous solutions of the resin or plastic in chloroform. We have used damar, shellac, rosin, and methyl-methacrylate plastic (Plexiglas). A disadvantage of rosin is its dark color which obscured a view of the tissue. Also it seemed a little too brittle during sectioning. We have had trouble with shellac. It has produced some excellent blocks, but at other times it has not hardened. If it proved important, no doubt this variability could be overcome. On the other hand, damar has been reliable in producing hard blocks, and is almost colorless so that the specimen can be seen clearly within the block.

Figures 2 and 3 are of human skin fixed with 2% osmic acid, and sectioned after embedding with damar. At the top of figure 2 the cuticular scales are essentially undisturbed, and sufficiently thin so that internal detail is visible. The lower part of figure 3 contains undisturbed dermis of a rather dense variety. We have had a great deal of experience trying to section skin during the past year, and there is no question in our minds but that the addition of resins improves the cutting quality of these blocks, and damar has proved particularly suitable.

Hair is perhaps the most difficult object we have tried to section. The various resins apparently do not adhere to hair very well, and the hairs always have loosened up in the block during attempts at cutting. We have only been able to section hair at room temperature when methacrylate was added to the block. Not only does this produce the hardest sort of block, but it becomes well bonded to the hair. Figure 1 shows one somewhat fragmented section of hair overlying another section. This overlapped view was selected deliberately to demonstrate the electron transparency of the sections involved, which in turn serves to indicate their thinness.

The triple embedding procedure has been tested on yet another difficult object, decalcified teeth. Some of the findings are the subject of an accompanying report (Pease, '51), and the illustrations there should be consulted. In teeth the hardness imparted to the block by methacrylate seemed essential.

Our use of plastic deserves special comment in relation to the embedding of Newman, Borysko, and Swerdlow ('49), who have used pure methacrylate. Their method entails polymerization which must be carefully controlled. When properly carried out, their technique undoubtedly yields a block with advantageous characteristics, and which can be cut. Our use of methacrylate in solution does not involve polymerization. Since only soluble fractions penetrate the nitrocellulose gel, solubility persists, and the plastic can be extracted totally without trouble. However we should not make any direct comparison of the two types of blocks for they are fundamentally different in character.

The idea of triple embedding sounds imposing, but actually is very simple, only requiring immersion in one additional solution. There are several intrinsic advantages in the method, for the characteristics of the block can be changed and controlled at will over wide limits. We have encountered one difficulty, however, in that slow physical changes sometimes have occurred which destroyed the cutting qualities of the block. Until a truly permanent combination can be found, blocks should be sectioned during the first week.

SUMMARY

Electron microscopy requires that the material being examined be very thin. Tissue sections usually can be no more than 0.1 μ thick. It has been possible to accomplish this, but only with some difficulty. A critical part of the process concerns the embedding medium which must give a relatively hard and unyielding block, and support the ultrathin sections. A new embedding technique is described which produces blocks with distinctly superior cutting characteristics. Material is first impregnated with nitrocellulose which is hardened by chloroform. This block is then soaked in a chloroform solution of a resin or plastic. Finally a molten wax bath is used which removes the chloroform solvent, but leaves the solute behind. Ribbons of 0.05 μ sections can be produced from such triply embedded blocks with but small refinements of usual technique, and a simple modification of a standard microtome.

LITERATURE CITED

FULLAM, E. F., AND A. E. GESSLER 1946 A high speed microtome for the electron microscope. Rev. Sci. Inst., *17:* 23–35.

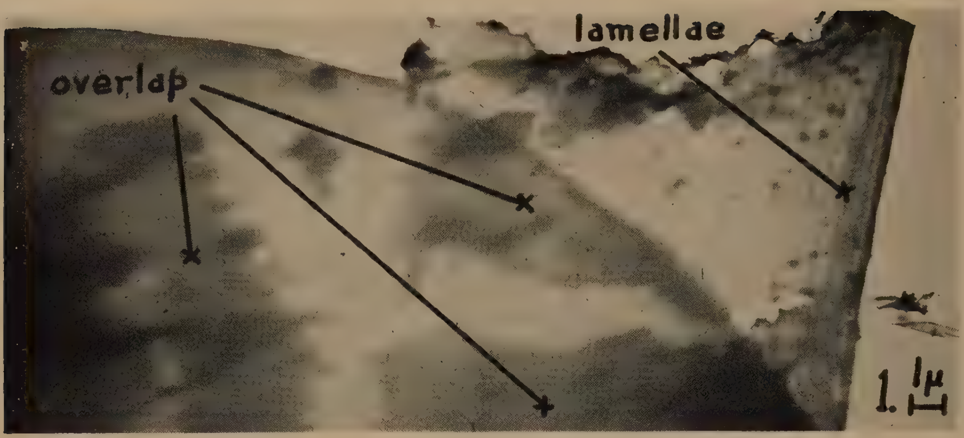
NEWMAN, S. B., E. BORYSKO AND M. SWERDLOW 1949 Ultra-microtomy by a new method. J. Research, U. S. Nat. Bur. Standards, *43:* 183–199.

PEASE, D. C. 1951 Electron microscopy of sectioned teeth. Anat. Rec., *110:* 539–547.

PEASE, D. C., AND R. F. BAKER 1948 Sectioning techniques for electron microscopy using a conventional microtome. Proc. Soc. Exp. Biol. Med., *67:* 470–474.

PEASE, D. C., AND R. F. BAKER 1949 Improved sectioning techniques for electron microscopy. J. Appl. Physics, *20:* 480.

——— 1950 Electron microscopy of the kidney. Am. J. Anat., *87:* 349–389.

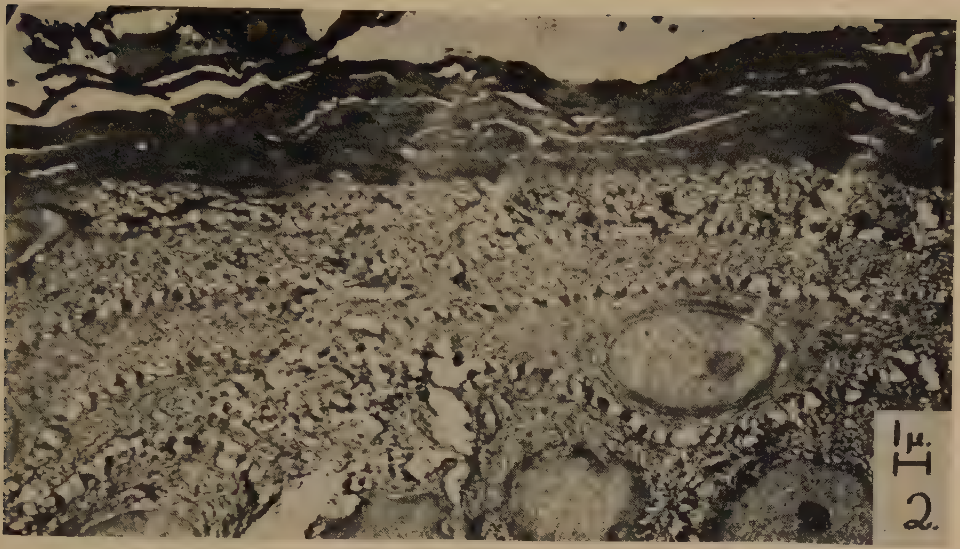


1 An electron micrograph showing portions of two sections of human hair superimposed. One section gives a good idea of the surface curvature. The other section is somewhat fragmented. Its circumferential lamellae show very clearly at the extreme right of the figure. Much of the rest of it overlaps the first section, and jagged tears can be seen through the latter.

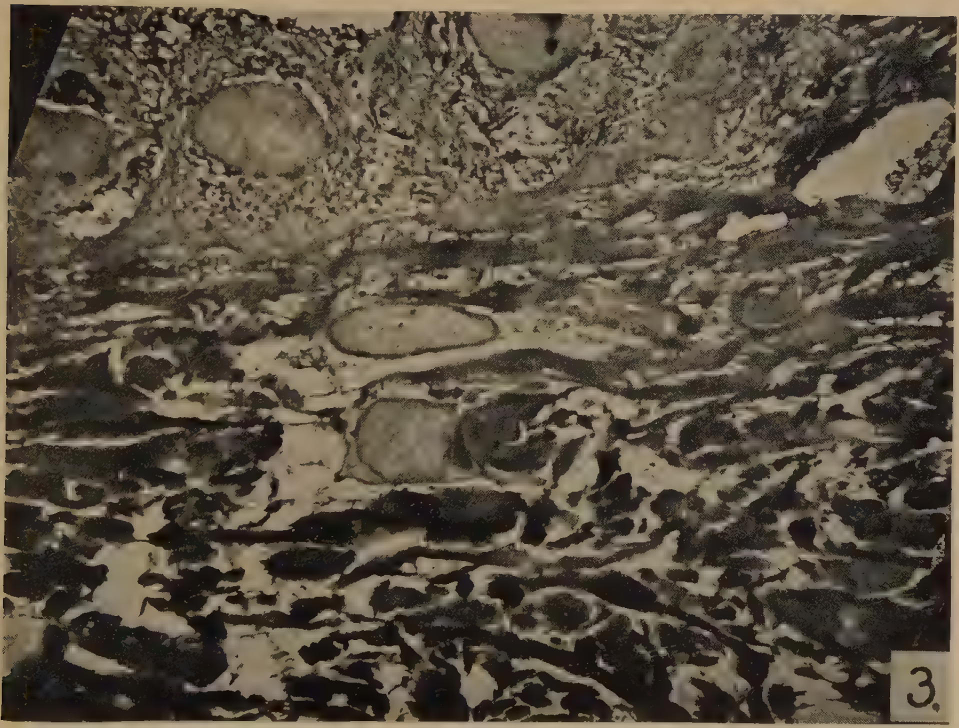
PLATE 2

EXPLANATION OF FIGURES

2 and 3 Transverse sections of human skin, from the back, close to the midline, and at a level approximately adjacent to the lower tip of the scapula. The cuticular layers are at the top of figure 2. The junction of epidermis and dermis is near the top of figure 3. Two fibroblasts lie in the center of figure 3.



2.



3.

ELECTRON MICROSCOPY OF SECTIONED TEETH

DANIEL C. PEASE[1]

Department of Anatomy, The Medical School, The University of Southern California, Los Angeles, California

FIVE FIGURES

Work in this laboratory has been directed towards improving methods of ultrathin sectioning for electron microscopy. This is the subject of an accompanying report (Pease and Baker, '51). These investigations have made it possible to work with decalcified teeth. Electron micrographs have been obtained which reveal new detail, and are discussed here. This work is complimentary to replica studies that have been carried on with considerable success in investigating enamel and dentine (cf. the review of Wyckoff, '49).

MATERIALS AND METHOD

The author's attention has been principally upon the problems of sectioning, and no comparative study of different fixatives or of different modes of decalcification has been attempted. It is realized that eventually this will be necessary in order to draw final conclusions. The present work, then, is based upon a single method of preliminary treatment.

The upper incisors of rats were removed immediately after a blow on the head. The teeth were broken into segments no more than 3 mm long. These were placed in 2% osmic acid for approximately 24 hours. For two-thirds of this time they were carried around in a pocket to afford agitation. Some osmic acid did penetrate, for when finally decalcified and sectioned, the dentin proved to have a uniform dark brown color.

---

[1] Present address, Anatomy Department, The Medical School, The University of California at Los Angeles, Los Angeles, California.

After fixation the teeth were washed with several changes of 50% alcohol, and then soaked in a half-saturated alum solution. They were decalcified according to the method of Fish as given in Lee ('37). This entailed using 5% nitric acid in half-saturated alum. The alum presumably served to prevent the swelling and dissolution of collagen in the acid. During the decalcification the vials again were carried around in a pocket. It was judged that 24 hours was sufficient time for this, but the knife was dulled so quickly during subsequent sectioning that decalcification may not have been completed. The acid was removed by a number of changes of half-saturated alum solutions over a period of several days. Finally dehydration was begun.

The details of embedding are the subject of an accompanying article (Pease and Baker, '51). Sectioning proved difficult, but principally because the knife dulled after a dozen or so sections had been cut, and needed readjustment. Thus it was not possible to obtain long ribbons, and the percentage yield of good sections was low. It was not so low, however, as to be impractical. As indicated, there may have been an abrasive action from incomplete decalcification.

FINDINGS AND CONCLUSIONS

Dentin probably presents the most interesting structural problem that might be studied by the electron microscopy of sectioned teeth. This is because both the organic and inorganic components are so finely divided as to be beyond the resolving power of the light microscope. Although a good deal is known about their arrangement (c.f. the review of Lehner and Plenk, '36), this depends upon indirect information such as can be obtained from birefringence studies.

In dentin some electron micrographs revealed an elaborate fiber structure as shown in figure 2. Other areas of the dentin have looked essentially amorphous as in figure 5. It is believed that these differences reflect either variations in fixation or decalcification. It is thought that fiber structure is present originally, but that there is difficulty in preserving it without gelatinization.

The fiber structure is of an altogether smaller order of magnitude than could be visualized with the light microscope. In figure 2, for instance, even the largest bundles are but slightly over 0.1 µ thick. The filaments of which they are composed are much finer. A beaded appearance is common, and cross linking seems to occur from one bead to another. However, a detailed description of these relationships would be unjustified at the present time.

It is generally believed that the predominant fiber axis varies in different parts of the tooth, and is not particularly related to the axes of tubules. It is obvious in figure 2 that the principal axis can, in fact, make an acute angle with the tubules.

It is commonly stated that the fibers of dentin are collagenous in nature. They have similar solubility characteristics. Schmitt and his coworkers ('42) discovered, however, that collagen appeared transversely banded in electron micrographs. The characteristic periodicity has not been seen in the electron micrographs of dentin. This may reflect the mode of preparation, and should not be regarded as strong evidence against collagen.

When the fiber structure has been preserved most perfectly, the tubules have seemed irregularly expanded (fig. 2). Thus exposed fibers have a tendency to contract. Tubules dimensions seem best preserved when the fiber structure barely shows, as in figures 3 and 1. Figure 3 is of a section much too thick to show detail in the dentin, but the transverse sections of dentinal tubules are obvious. Each has a somewhat shrivelled core representing the odontoblast process (also visible longitudinally in fig. 2). In figure 1, density differences are not as great, and the tubules are less conspicuous. The odontoblastic processes show with but little evidence of shrinkage in some tubules (circled). It was known that the section of figure 1 came from close to the surface. The tubules, therefore, were smaller than average, and were substantially less than 1 μ in diameter.

The upper part of figure 1 is of an excessively thin portion of a tapered section. (Density differences have been minimized during the photographic enlargement.) Small cavities may be seen clearly all over this area. They are rarely over 0.1 μ in diameter. It is tempting to think that they represent spaces left after the dissolution of salt crystals, and therefore reflect the size of individual crystals. Too many factors may have introduced artefacts, however, to accept this with confidence at the present time.

The sections through the pulp cavity show obvious chromatin granules in the cell nuclei. This is not a good sign, since these usually accompany an unduly vacuolated cytoplasm, and suggest inadequate fixation. This problem is discussed at length by Pease and Baker ('50). In figure 4, therefore, the vacuolated appearance of the cells may not have significance. The superficial layer is highly cellular. Cell membranes are present, but can be picked out only with difficulty for they are extremely thin, and without visible indications of supporting structure.

Central portions of the pulp show in the lower portion of figure 4. Cells are sparse. The interesting feature here is the almost total lack of extracellular fiber which one expects to see in a connective tissue. The only obvious fiber that has been seen so far has been associated with relatively large blood vessels.

Figure 5 is of a rather poor section from a technical point of view, but it offers conclusive evidence that odontoblastic processes extend into the dentinal tubules. In this junction region, fixation probably was better than deep in the dentin, so it seems significant that the processes fill the tubules completely.

In conclusion, it is obvious that ultrathin sections of teeth can be obtained which will yield good electron micrographs.

It will be possible to pursue a wide variety of problems in dental research. An extended study of the structural characteristics of dentin would seem particularly promising at the present time. However, this should be coupled with efforts to improve fixation and decalcification procedures.

SUMMARY

It has proved possible to make ultrathin sections of decalcified rats' teeth so that satisfactory electron micrographs have been obtained. Emphasis has been upon the technical problems of sectioning, but some structural details of teeth have been seen with a new clarity. These are described. Of greatest interest, probably, is the fibrous matrix of dentin. Fine bundles of fibrils are only about 0.1 μ thick. These are oriented parallel with one another, but make an acute angle with the axes of dentinal tubules. Under some conditions, minute cavities are to be seen in the dentinal matrix. These measure 0.1 μ or less in diameter. They may represent vesicles left after the dissolution of inorganic crystals, but the extent of artefact cannot be assessed at the present time. Odontoblastic processes can be traced into dentinal tubules, and at times completely fill their lumen. The most interesting feature of the pulp is its lack of fibrous structure. Distinct fibers have been seen only in relation to the largest blood vessels, and thus the pulp is by no means a typical connective tissue. Work to date has been confined to a single mode of fixation and decalcification. Other techniques must be explored in this new approach to dental research.

LITERATURE CITED

LEE, B. 1937 The Microtomist's Vade-Mecum (10th Ed.), Blakiston Inc., New York.

LEHNER, J., AND H. PLENK 1936 Die Zähne, Handbuch d. Mikroskop. Anat. d. Mensch (ed., W. von Möllendorff), *V/3:* 449–675.

PEASE, D. C., AND R. F. BAKER 1950 Electron microscopy of the kidney. Am. J. Anat., *87:* 349–389.

———— 1951 Triple embedding for the ultrathin sectioning required by electron microscopy. Anat. Rec., *110:* 531–538.

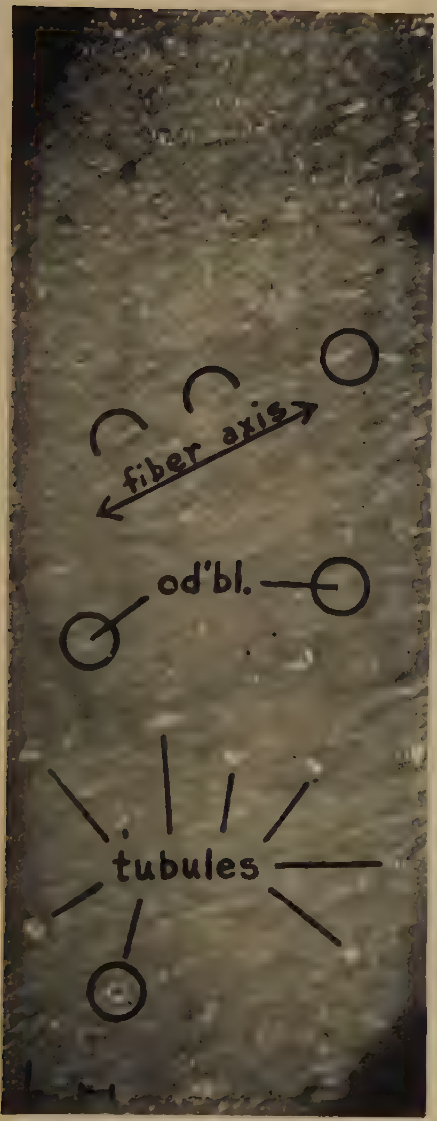
SCHMITT, F. O., C. E. HALL AND M. A. JAKUS 1942 Electron microscope investigations of the structure of collagen. J. Cell. and Comp. Physiol., *20:* 11–33.

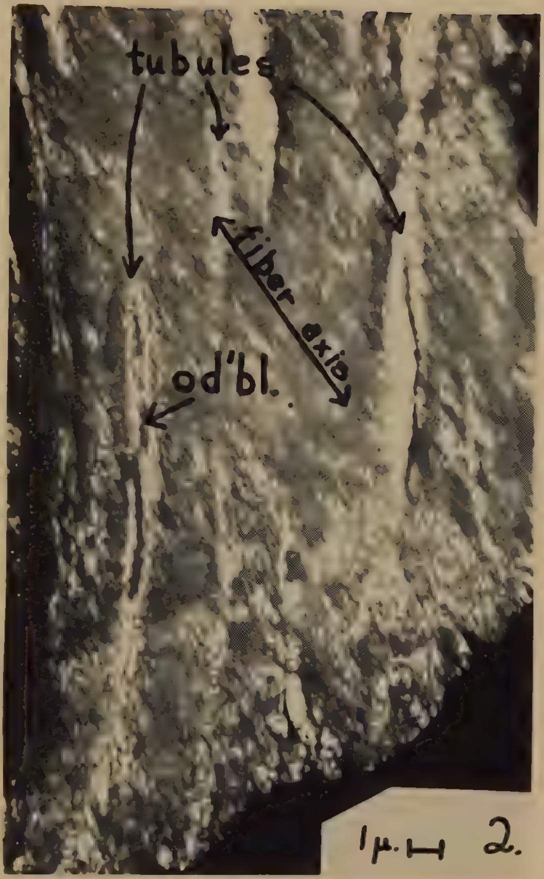
WYCKOFF, R. W. G. 1949 Electron Microscopy. Interscience Pub., New York.

PLATE 1

EXPLANATION OF FIGURES

Electron micrographs of decalcified dentin. The plane of section was parallel to the dentinal tubules in figure 2, and transverse to them in figures 1 and 3. The processes of odontoblasts *(od'bl.)* can be seen within dentinal tubules. Some tubules are circled which contain odontoblastic processes showing a minimum of shrinkage. The upper third of figure 1 is of a substantially thinner portion of the section than the remainder. Density differences have been equalized in the photographic printing. Micron marks indicate magnification.





2.

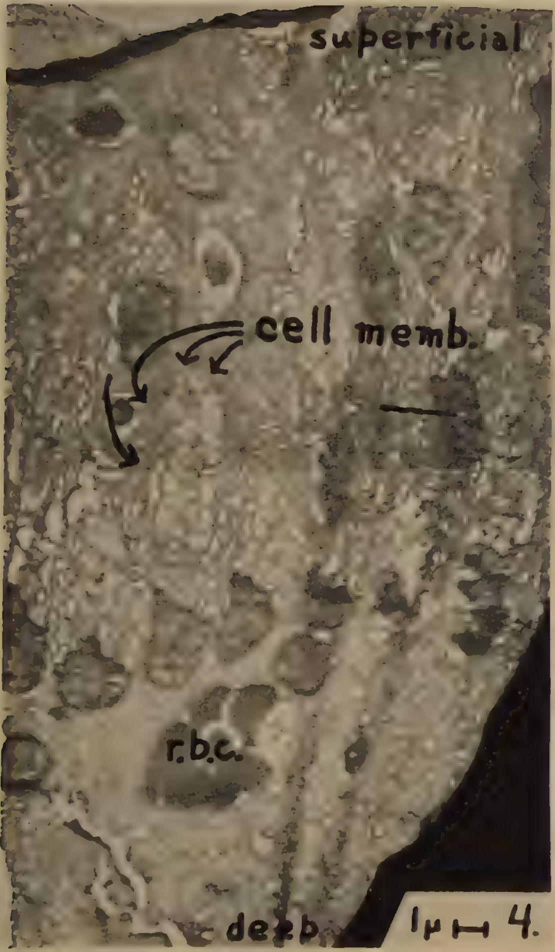
3.

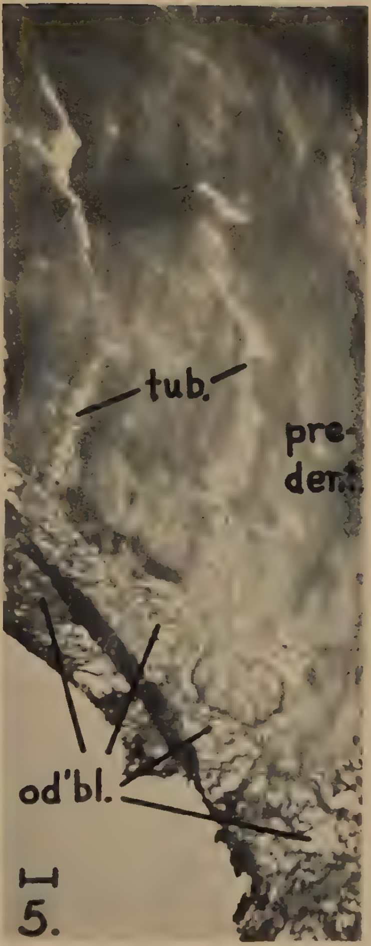
PLATE 2

EXPLANATION OF FIGURES

4 Part of a transverse section through the pulp cavity. The upper portion of the section had been torn away from dentin. The odontoblasts have such thin walls *(cell memb.)* that the cellularity is difficult to observe. The deep pulp in the lower portion of the figure is largely acellular. A small blood vessel with erythrocytes *(r.b.c.)* shows.

5 Junction of pulp and predentin. Odontoblasts *(od'bl.)* have processes penetrating and filling the dentinal tubules *(tub.)*.


superficial
cell memb.
r.b.c.
deep
1μ
4.


tub.
pre-
dent.
od'bl.
5.

CLEAVAGE LINE PATTERNS OF THE CAT

JOSEPH H. GARDNER AND HARRY E. RAYBUCK
Department of Anatomy, St. Louis University School of Medicine, St. Louis, Mo.

THREE FIGURES

In 1831, Duyputren, a French physician, called upon to treat a patient who had attempted suicide with a round bladed stiletto, reported to the police that the stiletto could not have been the potential lethal weapon since he observed that the resulting stab wounds were linear instead of circular. His curiosity having been aroused, Duyputren went to the laboratory where he found that linear shaped wounds could be produced in the skin of a cadaver by piercing it with a conical instrument. He also noted that the direction of the linear wounds varied in different regions of the body.

Thirty years later, Langer (1861) published the first anatomic account of cleavage line patterns. Kocher (1892) was the first surgeon to recognize and appreciate the surgical implications and the importance of cleavage lines. As a result of his work, they are sometimes referred to as Kocher's lines.

Cox ('41) investigated cleavage line patterns of man and reported as follows:

1. By subjecting a series of cadavers in the laboratory to over 22,000 puncture wounds made by a conical instrument the existence of lines of cleavage has been confirmed beyond a doubt.
2. Cleavage line patterns show some variation according to body build, but they are constant for bodies of similar build.
3. Cleavage lines are due to the arrangement of the collagenous and the elastic fibers. He also found, by puncturing excised skin that the connective tissue fibers were arranged parallel to the linear wound.

The present investigation was undertaken to determine, if cleavage lines exist in the cat, what type of pattern is present, and to obtain additional evidence that such lines are determined by the arrangement and the distribution of the connective tissue fibers.

MATERIALS AND METHODS

A total of 7 cats (one kitten and 6 adult cats) were used. Of these, 4 were females and three were males. The hair was removed and puncture wounds were produced by a metal probe of large diameter that had been ground to a sharp conical point. The wounds were painted black and photographed.

In order to determine whether fixation had any effect, both freshly killed and formalin fixed animals were used. No difference in the production or the pattern of cleavage lines was found in comparing the formalin fixed animals with the freshly killed one.

To corroborate the gross anatomic evidence histologically, blocks of tissue containing the linear wounds were removed and sectioned in three different planes: (1) the vertical plane parallel to the long axis of the wound, (2) the vertical plane at right angles to the long axis of the wound, and (3) the horizontal plane. The sections were prepared with Masson's trichrome stain in order to study the relationship of the blue staining collagenous and the red staining elastic fibers to the long axis of the wound.

To obtain evidence that connective tissue distribution determines the cleavage lines, several factors had to be eliminated. First, the influence of the underlying muscle, and secondly the overlying epidermis. To eliminate the former, the skin was removed and then pierced with the conical instrument. To eliminate the latter, pieces of skin were placed in 1% acetic acid for 24 hours (in a refrigerator). The epidermis was then separated from the dermis and both were pierced separately.

OBSERVATIONS

Consistent patterns of cleavage lines have been observed in the skin of all of the 7 cats that were studied and compared. These patterns (figs. 1–3) show that the long axes of the wounds run in different directions in different regions, but the pattern of these regions never varied in any of the animals. In some regions, most notably in the vental surface of the thoracic, the interscapular region, the sacral region and to a lesser extent the ventral neck region, the parallelism gives way to irregularly directed wounds. In these areas the wounds are not necessarily linear. Some of them tend to be triangular in shape. These areas appears to represent areas of unequal skin tension. Tension in the skin causes its retraction when it is incised. Human surgery has demonstrated that skin retraction is at a minimum when the incision follows cleavage lines, and at a maximum when the incision is made at right angles to the lines of cleavage. The lines of cleavage, therefore are in reality lines of tension.

Our histologic preparations confirm the earlier ones of Cox ('41). They present striking differences in structure depending on the plane of section. Sections taken parallel to the linear wound show a preponderance of collagenous and elastic fibers running parallel to the long axis of the wound. In the sections taken at right angles to the long axis of the wound the geat majority of fibers were cut transversely. The horizontal sections further confirm the direction of connective tissue fibers. Thus cleavage line patterns not only represent lines of tension but also afford an anatomical manifestation of the direction in which the majority of collagenous and elastic fibers run. The histologic data support the assumption that the cleavage lines are determined by the arrangement of the connective tissue fibers. In every case, it was found that when the skin was removed and pierced the cleavage line pattern remained the same as when the underlying muscle was present. When the epidermis and the dermis were separated and pierced separately, the wounds to the dermis were typical linear wounds, while those in the epidermis were definitely circular. The isolated epidermis showed no evidence of cleavage patterns.

DISCUSSION

Variations of the cleavage line patterns in the skin of man have been reported (Cox, '41, and Hutchinson, '51). Cox found that the patterns exhibit some variation according to body build, but these variations tend toward a constant for bodies of similar build. In the cat the patterns were found to be constant. This fact is explainable on the basis that in the cat there is very little variation in body type.

The factor or factors which determine the distribution of the superficial connective tissue are not fully known. The direction of the superficial muscle fibers may possibly influence the connective tissue distribution. This, however, appears to be doubtful since the cleavage line pattern does not correspond in every respect to the superficial muscle pattern. Since these lines in reality represent lines of tension it is more probable that the connective tissue distribution is influenced by muscle action. Gravity probably plays a part in the formation of cleavage lines. In comparing the cat (a quadriped) to man (a biped), a striking difference can be noted. In man, for example, the cleavage lines of the abdomen are transverse, while in the cat they tend to be longitudinal.

SUMMARY

Cleavage lines and cleavage line patterns are present in the skin of the cat. The cleavage line patterns are constant. This probably is correlated with the very small variation in body type. The linear wounds of cleavage lines are due to a preponderance of collagenous and elastic fibers running parallel with the long axis of the wound. There is little doubt that the connective tissue distribution in the dermis is responsible for the cleavage line since it was found that the lines are present in the dermis when it has been separated

from the underlying muscle as well as from the overlying epidermis. Cleavage line patterns represent tension line patterns and patterns representing the longitudinal distribution of the collagenous and elastic fibers in the superficial fascia.

LITERATURE CITED

Cox, H. T. 1941 The cleavage lines of the skin. Brit. J. Surg., *29:* 234–240.

Dupuytren 1834 Traité théorique et pratique des Blessures par Armes de Guerre, 2 volumes, 522, 527 pp., J. B. Baillière, Paris.

Hutchinson, C. T. 1951 Lines of cleavage in the skin of the newborn infant. Anat. Rec., *109:* 400.

Hutchinson, C. T., and P. T. C. Lam 1949 Lines of cleavage in the skin of newborns. Anat. Rec., *103:* 573.

Kocher 1892 Chirurgische Operationslehre. viii + 208 pp., G. Fischer, Jena. (Cited by Cox, '41.)

Langer, A. K. 1861 Zur Anatomie und Physiologie der Haut. Ueber die Spaltbarkeit der Cutis. Sitz. k. Akad. Wissen Wien., *44.* (Cited by Cox, '41.)

PLATE 1

EXPLANATION OF FIGURES

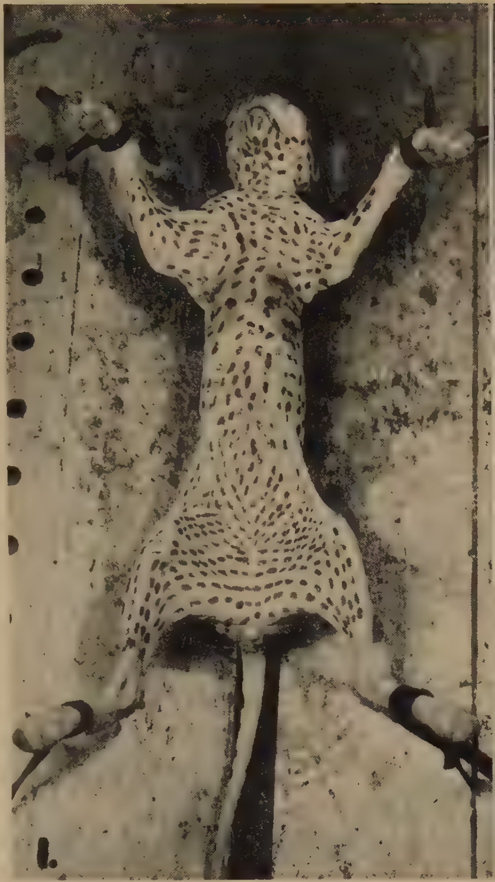
1 Ventral cleavage line pattern of the cat. Note the irregular ventral thoracic and neck patterns.

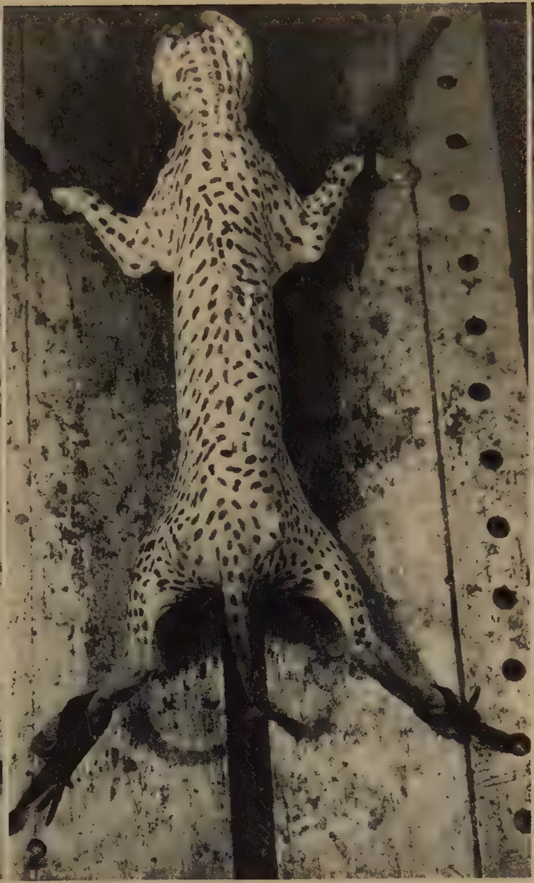
2 Dorsal cleavage line pattern of the cat. Note the irregular interscapular and sacral patterns.

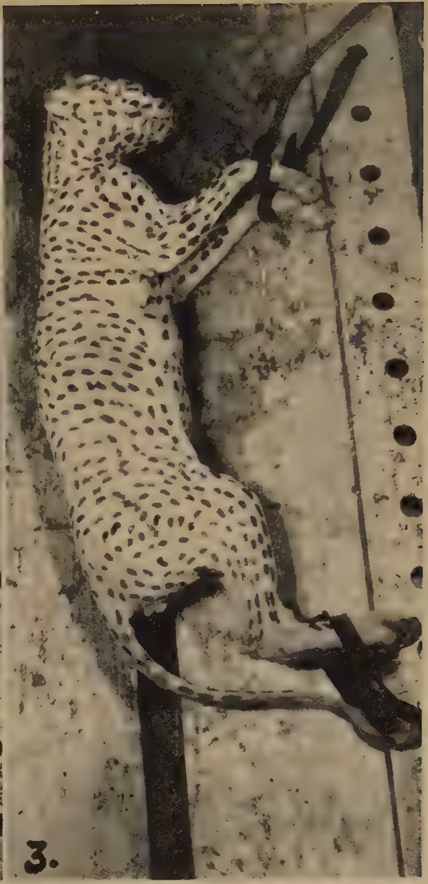
3 Lateral cleavage line pattern of the cat.

CLEAVAGE LINE PATTERNS OF THE CAT
JOSEPH H. GARDNER AND HARRY E. RAYBUCK

PLATE 1


1.


2


3.

EVIDENCES OF CYCLIC REPRODUCTIVE PHENOMENA IN THE RABBIT

CLARA EDDY HAMILTON
University of Georgia, Athens

EIGHT FIGURES

In mammalian species which have a clear-cut estrous cycle and, accordingly, reproduce only at certain times, there are cyclic alterations in the reproductive tract which are a reliable indication of ovarian activity and of cyclic variations in estrogen output. The rabbit, however, differs from such forms in that it has a continuous reproductive capacity under suitable conditions. This is dependent on the fact that ovulation does not occur spontaneously but occurs after a definite time interval (about 10 hours) following copulation. In the absence of ovulation in the rabbit, there is a series of follicles in all stages of development, with atretic follicles being numerous (Desaive, '48). Our problem has been to determine whether there are cyclic variations in the reproductive tract and the blood estrogen level of non-ovulating rabbits resulting from this ovarian activity. These questions have been studied by means of the vaginal smear, histological examination of the reproductive tract, and determination of blood estrogen levels.

Vaginal smear

METHOD

Vaginal smear studies were made of 6 rabbits for over 100 successive days. In addition, a number of studies were carried out for shorter periods of time while other phases of the work were being followed. The method of procuring the vaginal smear was to insert a smooth glass tube through the vestibule into the vaginal orifice and then to obtain a sample through this tube by means of a cotton-tipped applicator. This procedure prevented contamination of the sample with vestibular sloughage. Subsequent histological examination of the ovaries indicated that this method did not induce ovulation, since no evidence of existing or recent corpora lutea has been discerned.

The smears thus obtained were fixed in a mixture of equal portions of ethyl ether and 95% alcohol and stained by the Shorr differential vaginal smear stain, using the modifications suggested by Svihla (Hartman, '44). Different cell types were observed and their relative abundance determined. No slide containing less than 100 cells, exclusive of leucocytes, was considered in the analysis of results.

OBSERVATIONS

A. Cell types. A description of certain cell types that were regularly and frequently encountered in microscopic observation follows.

1. Large, cornified, non-nucleated scale type cell that stained a reddish orange with the Shorr stain.
2. Large, nucleated epithelial cells which showed 4 staining reactions: (a) red-staining cytoplasm with small, darkly-staining, compact nucleus; (b) red-staining cytoplasm with pale, vesicular nucleus; (c) green-staining cytoplasm with small, darkly-staining, compact nucleus; (d) green-staining cytoplasm with pale, vesicular nucleus.
3. Small nucleated epithelial cells which had a well-stained, vesicular nucleus and a cytoplasm of two staining reactions: (a) pale orange or reddish; (b) green.
4. Leucocytes.

B. Cyclic occurrence of cell types. A study of the relative abundance of the cell types, exclusive of leucocytes, was made by cell counts of the stained slides. It became apparent that certain cell types were more abundant at regular 4 to 6 day intervals, as is shown in figure 1. The cornified cells had spectacular and regular fluctuations of abundance, forming 80% or better of all cell types at the peak. In figure 2 is shown the frequency of abundance of the cornified cells in the 6 rabbits; in figure 3 a composite of the cornified cells in the 6 rabbits is graphed. The small red and green cells also had regular variations in abundance and at their peak often comprised 50% of the total cells present. Generally the peak of abundance of the small red cells lagged one or two days behind the small green ones. The large nucleated cells had fluctuations of abundance, but these were not consistent nor over a wide range. In the rabbits studied, leucocytes were only occasionally observed, and even more rarely were they abundant.

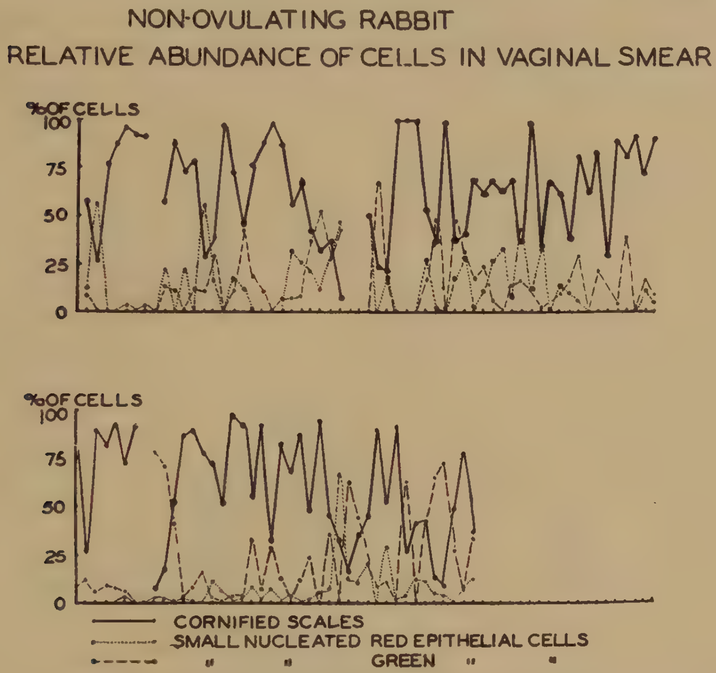


Fig. 1 Relative abundance of cornified scales, small nucleated red epithelial cells, small nucleated green epithelial cells in the vaginal smear of a representative animal.

Histology of the reproductive tract

METHOD

For this portion of the study 4 non-ovulating animals, two in the cornified phase of the vaginal smear cycle and two showing largely green epithelial cells in the vaginal smear, were used. After the animals were killed by air injection into the ear vein, the reproductive tracts were fixed in Bouin's and Zenker's fluids. Sections were made at 10 μ and stained

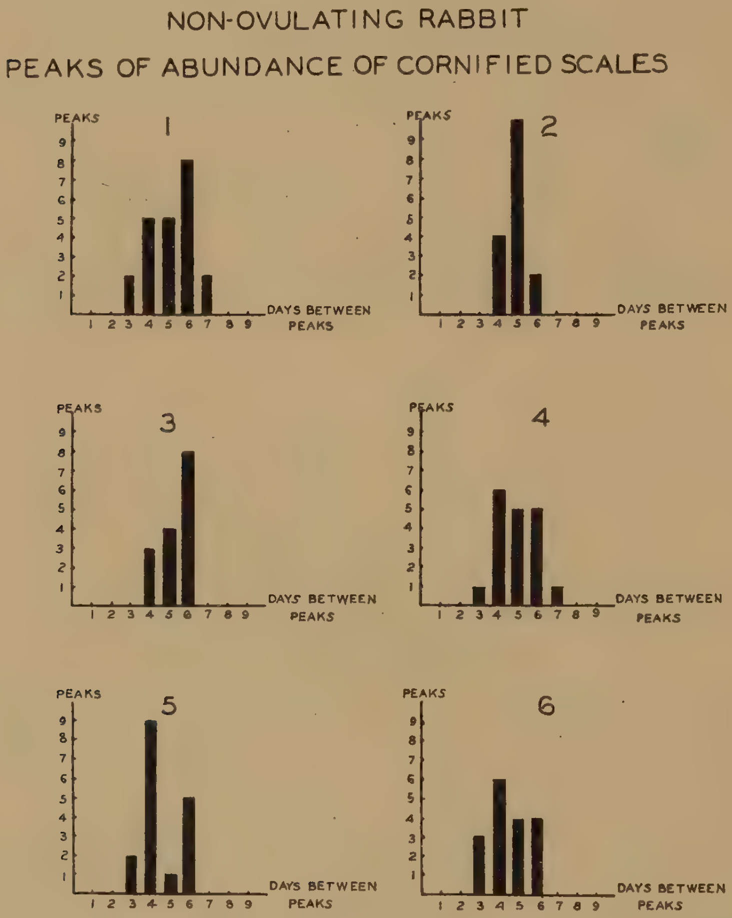
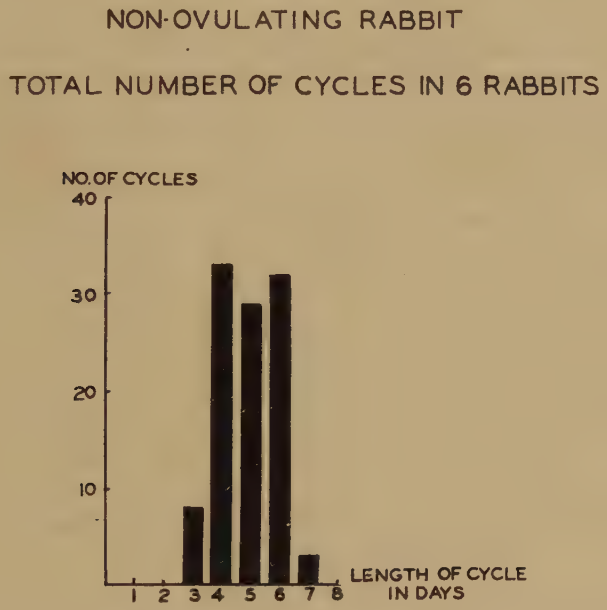


Fig. 2 Peaks of abundance of cornified scales in 6 non-ovulating rabbits.

in Harris's haematoxylin and eosin, Mallory's connective tissue stain, and Heidenhain's iron haematoxylin. Sections from all regions were also made at 5 μ and stained in the Shorr differential vaginal smear stain. The latter sections were of value in correlating changes in the mucosa of the reproductive tract with the vaginal smear picture and in relating the different staining responses of various regions of the reproductive tract to the cells seen in the vaginal smear (Hamilton, '47).


NON-OVULATING RABBIT

TOTAL NUMBER OF CYCLES IN 6 RABBITS

Fig. 3 Total number of cycles in 6 non-ovulating rabbits.

OBSERVATIONS

A. Ovary. The ovaries were characterized by abundant interstitial gland tissues, follicles in all stages of development and atretic follicles.

B. Fallopian tube. In the fimbria region of the oviduct there were long, finger-like projections of the mucosa. From anterior to posterior these folds became blunter, the tubal lumen increased, and the amount and compactness of the musculature increased. The entire oviduct was lined by a simple epithelium formed of tall, ciliated, columnar cells which were in close juxtaposition. In the fimbria region, cytoplasm of many of the cells stained a light reddish color with the Shorr stain, and any mucus in the cells stained green. In the body of the oviduct most of the lining cells had pale, green-stained cytoplasm that often contained red granules. Most of the cilia stained reddish, and there was a distinct terminal bar on the free end of the cells which stained a deep, brilliant red. The terminal bars were more pronounced in the Fallopian tube than in any other region of the reproductive tract. These results were uniform in the Fallopian tubes of all animals, regardless of the vaginal smear picture at the time of death.

C. Corpus uteri. The development of the uterine folds, glands, connective tissue stroma, and musculature and the extent of vascularization was that of normal, pre-ovulatory rabbits (Hammond and Marshall, '25). The mucosa exhibited slight variations in different regions. In the anterior region of the uterus, the lining epithelium was formed of tall, ciliated, columnar cells similar to the cells in the Fallopian tubes; posteriorly, the lining cells were rarely ciliated and were blunter, less crowded, and had more rounded nuclei. The glandular epithelium was formed of blunt, non-ciliated cells throughout the uterus. When red scales were predominant in the vaginal smear, the ciliated cells of the anterior portion of the uterus were green (Shorr stain); the lining epithelial cells of the rest of the uterus were stained red with an occasional interspersed green-stained mucus cell; the gland cells had red, lightly-stained cytoplasm; some sloughed red cells were seen in the lumen of the uterine canal; there was some evidence of secretory activity in the glands. When small, green epithelial cells were predominant in the vaginal smears, the uterus differed from the above description only in that the cells were slightly taller and the anterior ciliated cells had more red granules in their cytoplasm.

D. Cervix uteri. The mucosa of the cervix uteri (plate 1 A) resembled to a marked degree that of the upper oviduct region, having similar, tall, ciliated, closely crowded, columnar, epithelial cells lining the long slender folds. The characteristic convolutions of the cervix uteri were most numerous near the os cervicis. In comparison to the corpus uteri, the central lumen of the cervix was larger in diameter, the musculature was less compact and less prevalent, and the epithelial cells were taller, consistently ciliated, and more densely arranged. The transition from the uterine to the cervical type of epithelial cell was first seen on the larger folds. Many of the cervical cells had large vacuoles and are evidently similar to the cells in post-ovulatory and pregnant rabbits, as reported by Loeb and Smith ('35). The cervix apparently produced little mucus. When red scales were predominant in the vaginal smear, red and green staining, tall, ciliated, columnar cells were present and interspersed. When small, green epithelial cells were predominant in the vaginal smear, the cells lining the cervical canal and the cells in the gland crypts had green-staining cytoplasm which often contained red granules; both red and green staining cilia were seen; in the gland cells vacuoles were particularly noticeable, and the nuclei were flat, small, and parallel with the base of the cells. These cells are apparently identical to the mucus producing cells described by Hammond and Marshall ('25).

E. Vaginal fornix. The epithelium covering the projections of the cervix into the vagina and the epithelium of the vagina that surrounds these projections apparently underwent striking changes during the vaginal smear cycle. At those times when red scales were predominant in the vaginal smear, the cervical projections were covered with a shallow, stratified, squamous epithelium which stained deep red with the Shorr stain (plate 1 B); the vaginal recesses were lined with a thicker, stratified squamous epithelium which also stained a deep red and contained some superficial keratinized cells which perhaps were a source of the scales seen in the vaginal smear. When the vaginal smear contained an abundance of small, green epithelial cells, the cervical projections and the vaginal recesses were covered by a simple epithelium made of broad, non-ciliated, columnar cells that stained green with the Shorr stain (plate 1 D and E).

F. Vagina. The vagina had a smaller diameter at the vestibular end than at the anterior end. The mucosa was thrown up into broad, branched folds which were particularly large and numerous in the lower vagina. The lining was a simple epithelium with tall, broad, non-ciliated, columnar cells. When red scales were predominant in the vaginal smear, the lining epithelial cells were stained red while those in the gland crypts were green. At the times when the green epithelial cells were predominant in the vaginal smear, there were a few red-staining cells in the upper vagina, while the remainder of the cells stained green; the cells were actively secretory; the deeper gland cells were quite tall and had basal nuclei flattened against the attached surface of the cell.

G. Vestibule. The vestibule was lined with a thick, noncornified, stratified epithelium that resembled a modified transitional epithelium. With the Shorr stain this epithelium had a light, red-staining response, regardless of the vaginal smear picture. The color of the cells, the usual presence of nuclei, and our precautionary method of procuring the vaginal smear leads us to feel that this is not the "scale" seen in the vaginal smear.

Blood estrogen levels

METHOD

Hartman, Littrell, and Tom ('45, '46) developed a method for blood estrogen determination based on the fact that minute quantities of estrogen, when injected perivaginally, can cause opening of the vaginal closure plate in weanling rats and guinea pigs or reopening of it in the mature guinea pig. In our work, this method was modified by using ovariectomized guinea pigs as assay animals.

Forty-eight guinea pigs of comparable age and size were ovariectomized for use as assay animals. Attempts to establish a curve of known dosage of estradiol benzoate (Progynon B: courtesy of Schering Corporation) against time of response, such as was reported by Hartman and Littrell for the weanling rat ('45) were unsuccessful. Individual variations of response were too great for accurate information although there was a trend towards a more rapid opening at higher dosage levels.

In our blood estrogen tests, it was found that a priming dose of estrogen given prior to the test dose increased the sensitivity of the test. Three or 4 guinea pigs were primed with an injection of 0.01 cm^3 of estradiol benzoate (Progynon B: containing 1 mg per cubic centimeter) 7 days before receiving 0.05 cm^3 whole blood drawn from the ear of a rabbit (the same rabbit serving as donor for any one test series which lasted for 10 days). The priming dose was sufficient to cause increased vascularization and edema of the vulvar region. The injected animals were examined at frequent intervals and when any sign of activity was perceived the animal was examined hourly. After a three week rest period, an assay animal was reused.

TABLE 1

Summary of results of blood estrogen tests on 4 rabbits

NO. OF TESTS PERFORMED	NO. OF GUINEA PIGS TESTED	NO. OF ANIMALS RESPONDNG	NO. OF ANIMALS NOT RESPONDING	PREDOMINANT CELL TYPE IN SMEAR AT TIME BLOOD WAS TAKEN
8	25	16	9	cornified scales
7	25	7	18	green epithelial
3	9	4	5	red epithelial
8	23	3	20	mixture with none predominating

OBSERVATIONS

The tests as carried out did not determine quantitatively the blood estrogen level but did show that variations in the blood estrogen level occurred. The results, as given in table 1, indicate a higher blood estrogen level at the time that the vaginal smear contained a greater proportion of cornified cells.

DISCUSSION

In this study we have observed cyclic variations in the vaginal smear picture, in the histology of the reproductive tract, and in the blood estrogen level. In comparing the vaginal smear picture with sections of the reproductive tract, it was found that the alterations in structure of the tract are reflected in the vaginal smear picture. This was particularly true in the comparison of sections of the cervical and vaginal regions with the smears, for in these regions the histological changes are more striking. The vaginal smear probably represents sloughage from these areas as has been shown in the rat (Hamilton, '47). The blood estrogen level was found to be highest at the time of greatest cornification. The results of the blood estrogen studies were not quantitative which is in accord with the findings of Lloyd, Rogers, and Williams ('46), using the weanling rat.

It is known that the cyclic type of sexual phenomena in lower mammals and primates is a result of variations in the estrogen output, although the variations in estrogen output are not necessarily dependent on ovulation (Allen, Hisaw, and Gardner, '39). On the other hand, it has been shown that in normal or in castrate rats receiving a continued stimulation by a constant amount of injected estrogen (within a critical range) the reproductive tract also shows cyclic alterations which are, however, somewhat modified (Wade and Doisy, '35; del Castillo and di Paola, '42; and Hartman, '44). Therefore, there are two possible explanations for cyclic variations in the reproductive tract. There may be a cyclic estrogen output from the ovary which causes cyclic alterations in the reproductive tract or the reproductive tract may show cyclic variations in the threshold for response to estrogen. In this study, evidence from the blood estrogen studies indicates that there are possibly variations in the estrogen output.

Several observations were made that are at variance with results that might have been expected. In the vaginal smear, for example, leucocytes were only occasionally seen and even more rarely were they abundant. In many laboratory mammals a showering of leucocytes is a definite characteristic of certain phases of the vaginal smear cycle (e.g., mouse, Allen, '22; dog, Evans and Cole, '31, and others), and earlier investigators have reported abundant leucocytes in the rabbit smear but with no cyclic fluctuations (Snyder, '26; Kund and Proud, '29). The discrepancy between our results and the earlier findings in the rabbit may be due to differences in the methods for obtaining vaginal smears. Histological examination of the female reproductive tract did not reveal leucocytes crowding through the wall, as is seen, for example, in the vagina of the rat at metestrus (Long and Evans, '22). Another observation that is surprising is that the cervix of the rabbit produces only a small amount of mucus. This is unexpected as other species with a glandular-appearing cervical region (e.g., cow, Hammond, '25; monkey, Hamilton, '49; woman, Wollner, '36; and others) produce during the course of their reproductive cycle an abundance of mucus.

SUMMARY

1. The vaginal smear when free from vestibular sloughage and stained by the Shorr staining technic contained several cell types that recurred at 4 to 6 day intervals.

2. Thin sections of the reproductive tract stained by the Shorr stain indicated that the cervical and vaginal regions undergo cyclic alterations in their staining response which are correlated with the vaginal smear cycle.

3. Although the Hartman-Littrell blood estrogen method, using ovariectomized guinea pigs, has not proved to be quantitative in the modification used, the blood estrogen levels determined by this method indicate that there is an augmented estrogen output at the time the vaginal smear contained cornified cells in abundance.

LITERATURE CITED

ALLEN, E. 1922 The oestrous cycle in the mouse. Am. J. Anat., *30:* 296–371.

ALLEN, E., F. L. HISAW AND W. U. GARDNER 1939 Sex and Internal Secretions. Chapter VIII: The endocrine functions of the ovaries. 2nd ed. The Williams and Wilkins Company, Baltimore.

DEL CASTILLO, E. B., AND G. DI PAOLA 1942 Cyclical vaginal response to the daily administration of estradiol in castrated rats. Endocrinol., *30:* 48–53.

DESAIVE, P. 1948 Contribution à l'étude du mecanisme de l'évolution et de l'involution folliculaire dans l'ovaire de Lapine adulte. Arch. Biol., *58:* 331–446.

EVANS, H. M., AND H. H. COLE 1931 Oestrous cycle in the dog. Mem. Univ. Calif., *9:* 66–118.

HAMILTON, C. E. 1947 The cervix uteri of the rat. Anat. Rec., *97:* 47–62.

——— 1949 Observations on the cervical mucosa of the rhesus monkey. Carnegie Institution of Washington; Contributions to Embryology, *33:* 81–101.

HAMMOND, J. 1927 Reproduction in the Cow. Cambridge Univ. Press, London.

HAMMOND, J., AND F. H. A. MARSHALL 1925 Reproduction in the Rabbit. Oliver and Boyd, Edinburgh.

HARTMAN, C. G. 1944 Some new observations on the vaginal smear of the rat. Yale J. Biol. and Med., *17:* 99–112.

HARTMAN, C. G., AND J. L. LITTRELL 1945 A new test for blood estrogen. Science, *102:* 178.

HARTMAN, C. G., J. L. LITTRELL AND J. TOM 1946 The weanling guinea pig as test object for estrogen assay. Endocrinol., *39:* 120–130.

KUND, M. M., AND T. PROUD 1929 The ineffectiveness of vaginal smears in predicting the estrous cycle in the rabbit. Am. J. Physiol., *88:* 446–452.

LLOYD, C. W., W. F. ROGERS AND R. H. WILLIAMS 1946 Quantitative studies of the opening of the vagina of immature rats following injection of female blood and urine. Endocrinol., *39:* 256–260.

LOEB, L., AND M. G. SMITH 1935 The occurrence of cytoplasmic cell inclusions in the cervix of the uterus of the rabbit. Anat. Rec., *63:* 91–92.

LONG, J. A., AND H. M. EVANS 1922 The oestrous cycle in the rat and its associated phenomena. Mem. Univ. Calif., *6:* 1–148.

SNYDER, F. F. 1926 Ovulation in the rabbit. Anat. Rec., *32:* 242.

WADE, N. G., AND E. A. DOISY 1935 The prolonged administration of theelin and thelol to male and female rats and its bearing on reproduction. Endocrinol., *19:* 77–87.

WOLLNER, A. 1936 A preliminary study of the cyclic histologic changes of the human cervical mucosa in the intermenstrual period. Am. J. Obstet. and Gynecol., *32:* 365–374.

PLATE

PLATE 1

EXPLANATON OF FIGURES

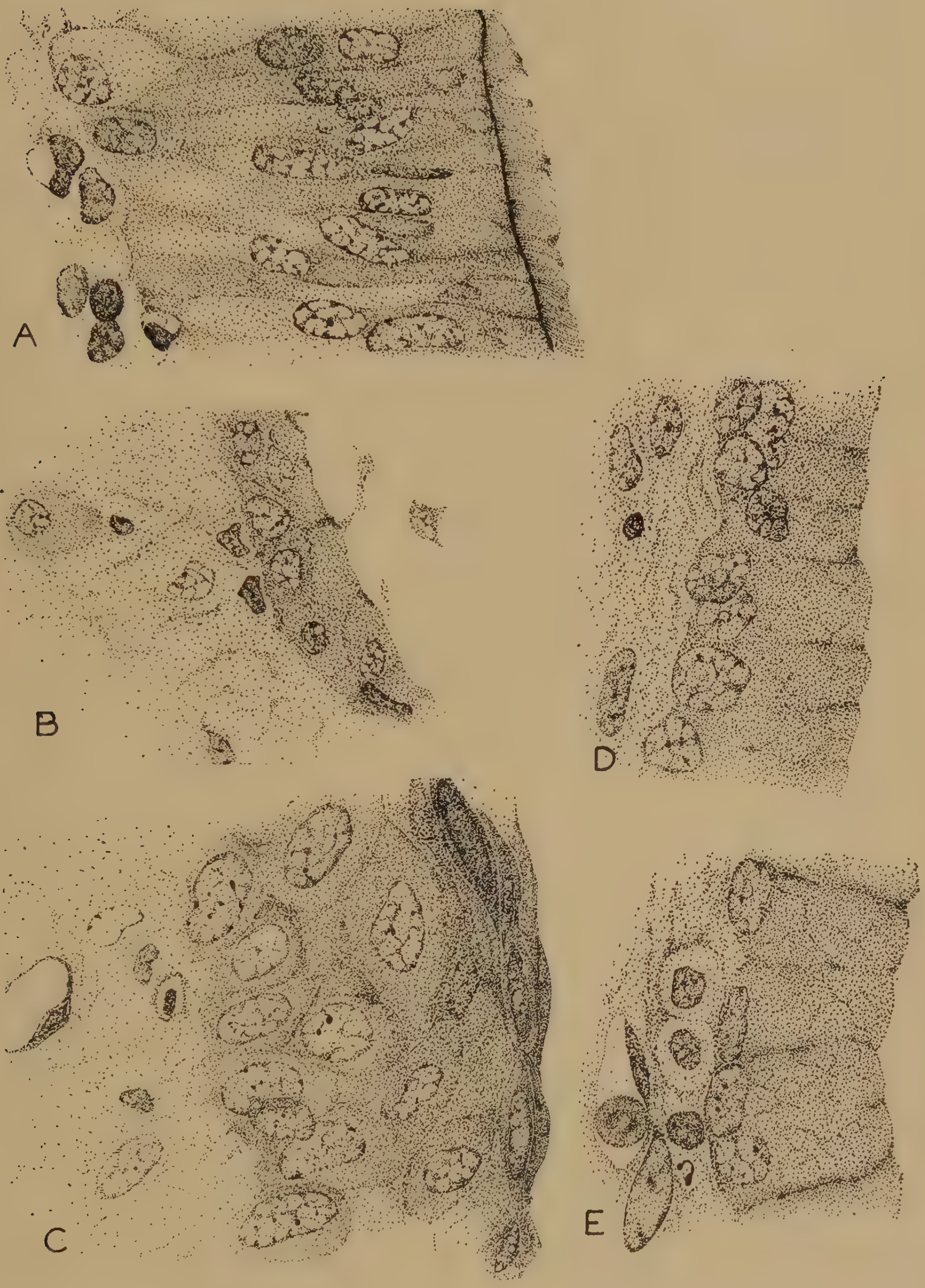
A Cervical mucosa during the time small green epithelial cells were abundant in the vaginal smear.

B Mucosa of the outer surface of the cervical projection during the time large cornified cells were predominant in the vaginal smear.

C Mucosa of the vaginal fornix during the time large cornified cells were predominant in the vaginal smear.

D Mucosa of the outer surface of the cervical projection during the time small green epithelial cells were predominant in the vaginal smear.

E Mucosa of the vaginal fornix during the time small green epithelial cells were abundant in the vaginal smear.


A
B
D
C
E

MORPHOGENETIC STUDIES OF THE RABBIT

X. RACIAL VARIATIONS IN THE GALL BLADDER [1]

PAUL B. SAWIN AND DORCAS D. CRARY
Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

TWO FIGURES

INTRODUCTION

Based upon relatively small numbers of observations, usually from individual dissections, mammalian species have been classified into those which do and those which do not possess gall bladders. Due to simplicity of structure and the functional importance of the gall bladder in metabolism, most investigations of this organ have been experimental. Only occasional references deal with morphological irregularities. Most conspicuous of the latter are those of duplication or of absence, but peculiarly enough, in the literature the absence of the gall bladder heretofore has appeared most significant as a species rather than as an individual difference. Gorham and Ivy ('38) assembled the literature and examined specimens from which they compiled a list of more than 800 species in which the nature of the organ is considered to be known. They state that "little effort has been made to describe variations in the form of anatomical relationships of the organ" and "that this hardly seems necessary since the organ is either present or absent and that no series between the extremes can be demonstrated." They conclude that the gall bladder is a primitive mammalian structure which is not easily lost and therefore of considerable stability, so much so in some orders as to

---

[1] This study is a part of a research program supported by grants from the Rockefeller Foundation, from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council, and the National Cancer Institute, U. S. Public Health Service.

be considered of taxonomic value. In general, forms which most closely resemble the ancestral type tend to retain the gall bladder, whereas those which vary most widely may lose it. Some closely related species, for example among the Muridae, may differ in the possession of the organ, and the occurrence of anomalous absences in individuals of some species suggest that it might be possible to study the mechanism of inheritance of the organ. *Oryctolagus cuniculus* is included among the species listed as possessing a gall bladder, which is in agreement with most authors who describe the anatomy of the domesticated rabbit.

The present communication records the variation in size of the gall bladder in the domestic rabbit, and thus, particularly the incidence of gall bladderless individuals observed in a species normally considered to possess such a structure. Since the major portion of the population observed is composed of some 25 unrelated races, several of which have been inbred for as many as 22 generations, the facts may be considered in terms of genetic influence and their possible use in genetic experiments, particularly those which concern regional growth and development of this organ and its function.

Because of the use of some of these races in previous genetic studies of regional growth forces, these observations assume significance from the viewpoint of function and of evolution.

OBSERVATIONS

Credit for the origin of this communication rightly belongs to Mr. George Lilyestrom of Paxton, Massachusetts, whose careful observations, interest and generosity first brought the variation to the attention and hands of the senior author, and made possible the establishment of a race known as *Ly*, in which such variates are found to occur with a certain degree of regularity (see line 19, table 1). Not only has it been found that rabbits belonging to this race of New Zealand Whites repeatedly produce offspring which lack a gall bladder, but when the study was extended to include all the races in our colony it was found that the absence of a gall bladder is part of a graded series of variations in its size and shape.

Size. Because the size of the gall bladder in the beginning of the study was a consideration secondary to its presence or absence, and because of the heterogeneous nature of the material with respect to both age and adult body size, the post

TABLE 1

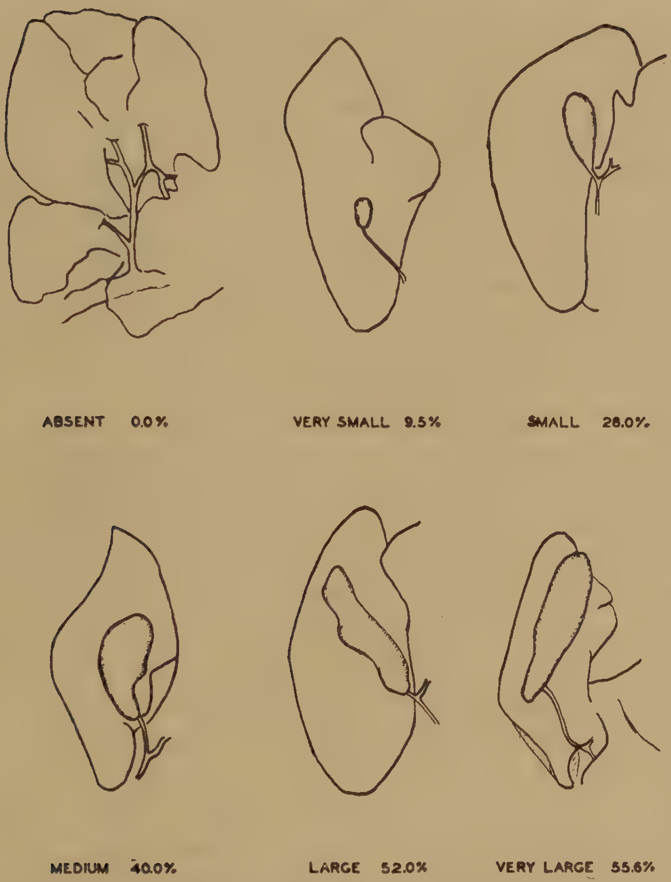
Per cent distribution of variations in gall bladder size in 25 inbred races of rabbits and one population of miscellaneous origin

GROUP	RACE	NO. OBS.	NONE	VERY SMALL	SMALL	MEDIUM	LARGE	VERY LARGE
			(0)	(1)	(2)	(3)	(4)	(5)
I	Polish (1)	37	..	2.7	59.5	32.4	5.4	..
	X (2)	184	1.1	12.5	57.1	25.3	3.3	..
II	Hyde (3)	61	..	..	3.3	54.1	34.4	8.1
	Kd (4)	68	..	..	11.8	61.8	23.5	2.9
	Rous (5)	50	..	4.0	32.0	44.0	20.0	..
	Ac (6)	173	..	1.2	24.2	50.1	20.2	4.1
	Os (7)	71	..	2.8	35.2	38.0	23.9	..
	Ax (8)	192	..	13.5	35.3	40.6	10.4	..
	V (9)	72	..	23.6	44.4	25.0	7.0	
	R (10)	27	..	..	37.0	55.6	7.4	
	DRD (11)	79	..	15.2	57.0	24.1	3.8	
	Av (12)	40	..	7.5	60.0	22.5	10.0	..
III	TC (13)	52	..	..	1.9	69.2	25.0	3.8
	III (14)	84	..	1.2	6.0	50.0	34.5	8.3
	IIIc (15)	296	..	..	13.4	58.1	24.8	3.4
	Wwd (16)	73	..	4.1	17.8	53.4	21.9	2.7
	Smith (17)	80	..	11.3	37.5	37.5	13.8	..
	Barone (18)	42	..	..	14.3	45.2	33.3	7.1
	Ly (19)	99	10.1	30.3	32.3	25.3	2.0	..
	Da (20)	38	2.6	..	34.2	50.0	13.2	..
	HS (21)	60	..	..	18.3	55.0	21.6	5.0
	BrF (22)	146	..	2.7	39.0	54.1	2.7	1.4
	Belgian (23)	35	..	..	31.4	54.3	14.3	..
IV	S. Flemish (24)	73	1.4	2.7	37.0	50.7	8.2	
	Ep (25)	73	..	9.6	41.1	41.1	8.2	
V	Misc. (26)	1275	.58	8.0	37.6	42.5	10.6	.9
	Total (27)	3480	.6	6.9	32.8	44.4	13.8	1.6

mortem observations being made upon animals dying or being sacrificed for one or another of several experimental purposes, evaluation of size was first made arbitrarily on the basis of 6 classes, including the complete absence. Because all bladders develop in relation to the liver, and the liver, with one possible exception to be discussed later, seemed to show development in proportion to the size and age of the animal, this classification has been placed on a quantitative basis by expressing the length of bladder in per cent of the length of the lobe to which it is attached. Measurements were usually made on the fully distended organ. Some emptied bladders were noted and the collapsed and somewhat wrinkled nature indicated that empty bladders are not reduced in size, as is the empty urinary bladder, and that measurements of length are therefore reliable. Representatives of each class are shown in figure 1, with class ranges as indicated below.

The total population, consisting of 3480 animals, observed during 1949 and the first 8 months of 1950 has been separated into 5 groups as shown in table 1. In all, 53 genetically different populations have been examined including inbred races and hybrid generations of various kinds. Since, during the time in which this material has been collected, particular emphasis has been placed upon study and development of inbred races, the major portion of the data have been derived from this source. Only those races are shown separately which have produced 25 or more progeny. The remainder, together with hybrids of various types, and miscellaneous matings made for other specific genetic studies where numbers are small, have been combined and considered as a general or control population (group 5, table 1). In this population the distribution is very nearly symmetrical, but slightly skewed to the right, indicating a slightly greater proportion of large gall bladders than would be expected by chance.

The 25 pure races have been arranged according to mean adult body size and separated into 4 groups, the members of each group being of about the same body size. Group I contains the Polish and race X (an inbred race of Polish extrac-



ABSENT 0.0%
VERY SMALL 9.5%
SMALL 28.0%
MEDIUM 40.0%
LARGE 52.0%
VERY LARGE 55.6%

Fig. 1 Variation in size of gall bladder, classified according to per cent of length of right anterior lobes of liver. Per cents shown apply to the particular specimens shown. The range of size for each class is as follows:

very small 0.1–25.0
small 25.1–35.0
medium 35.1–45.0
large 45.1–55.0
very large 55.1 plus

The largest observed has been 103.1%.

tion, originally descended from the small race of Castle) whose mean adult body sizes are 1800 and 2200 gm respectively. Group II is a group of races of varying origin but with a mean adult body weight of approximately 3000 gm.[2] The first 8 races of group III are of New Zealand White origin. They have been obtained from laboratories or breeders for some specific genetic reason and all of which so far as is known, are entirely unrelated, except the first three which are sublines of the same race. The HS race was derived from a Himalayan-New Zealand White cross, and the BrF is a synthetic stock of miscellaneous origin. The Belgian is one of the oldest races in this country and of particular interest for its slender proportions and refinement of bone. All of these races weigh approximately 4 kg. Group IV is a group of extreme body size or proportion including the Sandy Flemish with a mean adult body weight of 6.5 kg, and the Ep line which is of Flemish extraction.

---

[2] The Ac, Av, Os and DRD races are stocks derived from races formerly maintained by the Rockefeller Institute, Princeton, New Jersey.

There appears to be little, if any, evidence of correlation between adult racial body size and the size of gall bladder. Animals with no gall bladders appear in race X (group I), races *Ly* and *Da* (lines 19 and 20, group III, table 1) and in the Flemish (group IV). Only in group II do we fail to find individuals which completely lack gall bladders, but it is also interesting to note that whereas most of the gall bladders of group II animals tend to be of good size, race V has almost 25% of individuals with very small gall bladders.

With respect to the other extreme, very large gall bladders are not found in the races of either group I or group IV, races of either extreme of adult body size. They are found in greatest numbers in the two intermediate groups, but at the same time they are not found in all the races of either of these two groups.

Further, examination of the distribution of the variations in size among the various races in general shows them to be continuous, and in most cases nearly symmetrical or slightly skewed in one direction or the other. In the R race (group II) and the Belgian (group III) the variation is concentrated in three classes (small, medium and large) and the distribution is but slightly skewed toward small size. All other races, in which the number of observations are larger, are distributed in at least 4 classes. In races KD, and Hyde (group II), IIIc, Barone, HS and TC (group III) the distribution involves the

4 larger classes. Race X (group I), race Ac (group II), races III, Wwd, Ly, Da, BrF (group III) and Sandy Flemish (group IV) are distributed in 5 classes.

Of these, in III, Ac, Wwd, and BrF, the distribution involves the upper classes, all individuals having a gall bladder ranging from very small to *very* large, whereas in X, Ly and Sandy Flemish it involves the lower classes, including animals with no gall bladder at one extreme and large gall bladders at the other.

At this point it becomes of interest to know whether the relative size of the bladder is influenced by age. To test this possibility 693 animals from the control population, classified at birth, have been compared with the remainder classified from two to three months to several years of age. The difference is highly significant as tested by chi-square and examination of the two populations shows that the average newborn bladder is about 0.3 of a class smaller relatively than that of the older age group.

This difference is not entirely the result of the age at classification, however, because when a similar comparison was made within the 6 largest racial populations (see table 2) in two of the races, IIIc and BrF, the differences between old and newborn populations are found not to be significant. These happen to be races in which the average gall bladder is larger than in most of the races described, and in these apparently the gall bladder is already comparatively large at birth. In the *Ly* race, in which the gall bladderless condition was first observed, the difference lies just at the .01 level of probability showing that although newborn gall bladders tend to be relatively smaller in the general population it may not necessarily be so in all races. Races IIIc and BrF appear to achieve maximum gall bladder proportions at an early age.

Thus, it appears that the absence of a gall bladder is not an independent monogenic characteristic in itself but is a part of a graded quantitative series of variations, comparable to variations in ribs, vertebrae, sternebrae number and vascular pattern as described by Sawin and others (see Peck and Sawin, '50). Furthermore, from the differences in size in old and young populations of the several races, there is reason to suspect that the size of the gall bladder is a racial difference associated with differences in growth rate such that it may achieve its size earlier in some races than in others.

Shape and number. Variations of this kind are of about the same frequency as the absence of a gall bladder; there have occurred only 39 or 1.1% in the total population. However, in spite of the small numbers in this case, there is, as with size, a tendency for certain types to appear several times in the same race and to be absent in others. These anomalies have been classified as shown in table 3 and figure 2 with the racial incidence indicated.

TABLE 2

Per cent distribution of gall bladders of different size in sample populations of old and newborn rabbits in six races

POPULATION	NO. OBS.	NONE	VERY SMALL	SMALL	MEDIUM	LARGE	VERY LARGE	χ^2	P
X old	143	..	4.1	65.7	26.5	3.5	..		
newborn	41	7.3	41.4	26.8	21.9	2.4	..	57.6	>.01
IIIc old	208	..	..	12.9	54.3	27.8	4.8		
newborn	88	..	..	13.6	67.0	19.3	..	7.8	>.50
Ac old	98	..	..	12.2	54.0	27.5	6.1		
newborn	75	..	2.6	40.0	45.3	10.6	1.3	25.0	>.01
Ax old	97	..	..	32.1	48.2	19.5	..		
newborn	105	..	24.7	37.9	34.3	2.8	..	20.3	<.01
BrF old	67	..	..	38.8	56.7	2.9	1.5		
newborn	79	..	5.0	39.2	51.8	2.5	1.3	3.9	>.90
Ly old	23	..	60.8	39.1	..	..	..		
newborn	76	13.1	21.0	30.3	32.8	2.6	..	21.7	<.01

Of these types, type 4 (bifurcated or cleft, fig. 2G) and type 5 (bilobed or diverticular, fig. 2I) seem comparable to similar types described by Boyden ('26) which were found to arise embryologically by primary subdivision of the original bladder or by buds from the neck of the embryonic gall bladder and usually associated with cyst-hepatic ducts. Type 3 (diverticulate, fig. 2E and 2J) specimens are similar to those of the sheep

(Boyden) with diverticula occurring in body, neck and fundus. Such anomalies apparently arise from epithelial pearls or buds from any part of the embryonic gall bladder. Type 2 bladders (constricted, see fig. 2B, 2C and 2D) apparently are not common in other species but have been reported in man where they have been classified with the bilobed group (Deaver and Ashburst, '14). It is possible, however, that they are associated with irregularities in the primary vesicle which actually originates as little more than a hollow tube attached to an actively increasing mass of liver tissue. Only one case of ductular bladder (fig. 20) which is fairly common in cats, was observed, and this could have been of trabecular origin. Round

TABLE 3

Distribution of anomalies of gall bladder shape according to race

	RACE	TYPE				
		(1) Round	(2) Constricted	(3) Diverticulate	(4) Bifurcated	(5) Bilobed
1.	Polish	1	. .	. .	. .	. .
2.	X	4	. .	. .	1[+]	.
3.	Rous	2	.	. .	. .	.
4.	A	. .	. .	. .	1	. .
5.	Ac	. .	2	1	. .	. .
6.	DK	. .	. .	. .	1	
7.	R	. .	2	. .	. .	.
8.	III	. .	. .	4	. .	. .
9.	IIIc	. .	3	. .	. .	. .
10.	Wwd	1	. .	. .	. .	. .
11.	Smith	. .	. .	2	. .	. .
12.	Sp	2	. .	. .	. .	. .
13.	Barone	. .	. .	1	. .	. .
14.	Ly	2	.	. .	1	. .
15.	BrF	1	1	. .	. .	. .
16.	Belgian	1	. .	. .	. .	. .
17.	S. Flemish	1	. .	. .	. .	. .
18.	Kd × Rous	. .	. .	. .	. .	3
19.	Misc.	. .	. .	1	. .	. .
20.	Total	15	8	9	4	3
	Per cent	38.4	20.5	23.0	10.3	7.7

[+] = triangular in shape.

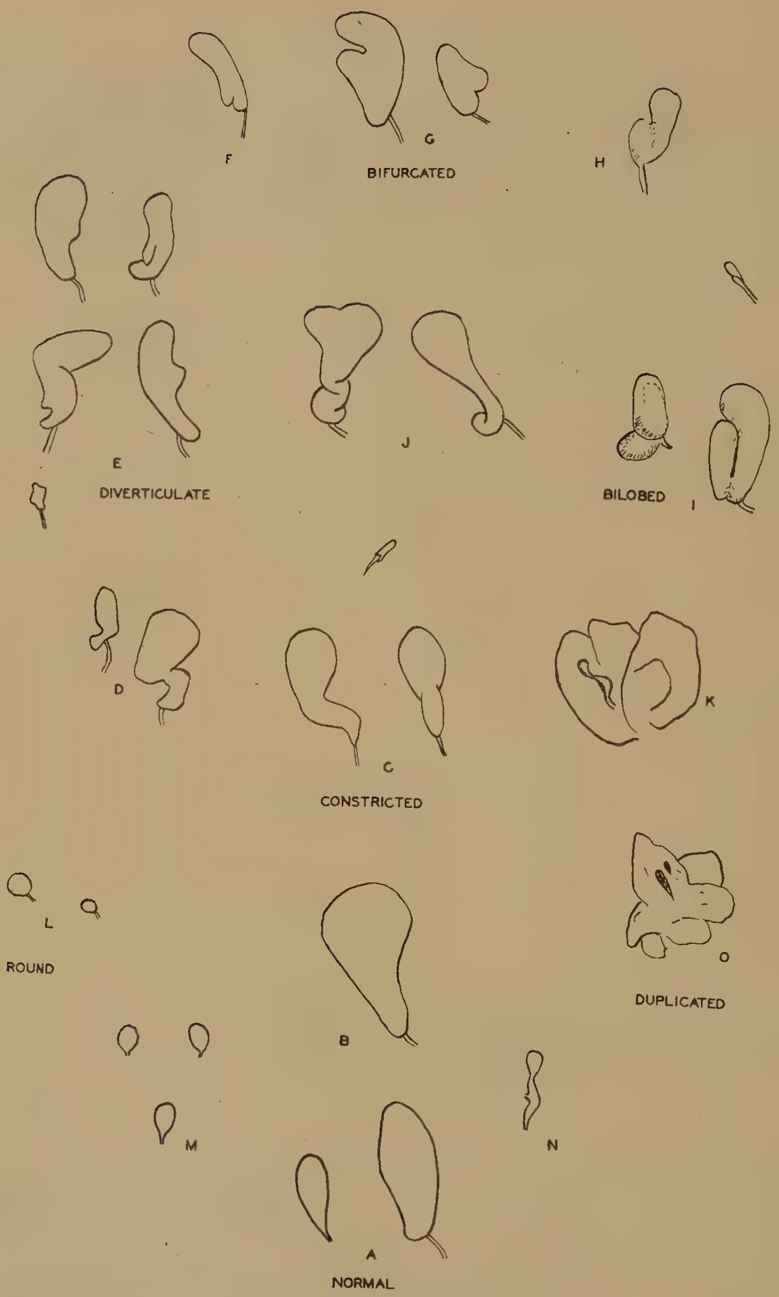


Fig. 2 Anomalies of shape of the gall bladder. Seven primary types are labelled with intermediate types so placed. It is possible that types C, D, and E, which are typical examples of the "jack-knife" type, the most common congenital anomaly in man, should be given a special designation (see Boyden, '35).

bladders (fig. 2L) are the most numerous anomaly of all in the rabbit, but apparently have not been described by other investigators. They seem to be more numerous in the races having the smaller gall bladders and thus may be assumed to be associated with restricted or localized growth. Figure 2 B, D, F, H, K, M and N are intermediate types.

The variations are distributed among 19 genetically different inbred and (one) hybrid races and seemingly without particular reference to adult body size. Race III is particularly conspicuous in having in the two sub-lines the largest number of anomalies and especially so because in race III^1 (see line 8, table 3) all are of the diverticulate type and in race III^c (line 9, table 3) they are all of the constricted type. The Rous (table 3, line 3) including its hybrids (line 18), is the only other racial group with more than three anomalies, and in the former the anomalies are of type 1, and in the latter of type 5. The KD race, thus far, has shown no anomalies of shape.

The incidence of neither of these variations, size or shape, is sufficient to throw much light on the exact mechanism of inheritance, but the fact that the bladder does fail to manifest itself in a substantial portion of individuals of a species commonly known to possess such an organ, together with the fact that it fails to appear much more frequently in some inbred races than in others, is sufficient evidence that it does have a genetic background. Further, the fact that this absence appears to be a part of a graded and continuous series of variations in size of the bladder suggests that the entire range of variation is in some way associated with the normal growth processes of the body, a concept which is also gained from study of the variations in shape, which will be discussed below.

DISCUSSION

These observations clearly indicate that the rabbit is an animal which usually, but not necessarily always, has a gall bladder, although heretofore it has been regarded as a species characteristically possessing one. One is led to question whether or not other species in which the number of observations is small may not likewise have been inadequately examined for proper classification. Since, as has been shown by Schmidt and Ivy ('37), the function of the gall bladder is variable between species, one may suspect either that it likewise may be highly variable within the species; or that the rabbit like the guinea pig is a species in which it seems to possess no essential function and hence is of little evolutionary significance. It must also be remembered that in man and the dog it has been shown that the gall bladder has both a storage and a pressure regulatory function. Yet Mentzer ('29) reports that congenital absence of the gall bladder has been reported in at least 50 patients and the incidence of this variation from the report of Nagel must be about one in 1600 individuals. Thus, even individuals of species in which the bladder is known to have a dual function apparently can get along without it, although according to Schmidt and Ivy there is no compensation so far as a change in bile concentration is concerned. They find that in cholecystectomy in the rabbit there occasionally results some dilation of the common duct, indicating that the gall bladder does exercise some pressure regulatory function. It is possible that the important difference is in the development of the sphincteric muscles, but this condition has not been examined as yet. Higgins ('26) suggests that there may be an intrahepatic compensatory mechanism in the gall bladderless rat for concentration of bile which does for this animal what the gall bladder does for the mouse, but in any case it would seem that the presence or absence of a gall bladder is of minor importance, either functionally or from the evolutionary standpoint. Either there is an extremely wide range of tolerance, so far as quantity and rate of delivery of bile to the intestine is concerned, or the inadequacy, resulting from absence of a gall bladder, is compensated in some other manner than those thus far considered and investigated.

As to the underlying reason for both variations in size and type, there is evidence that they could be due to regional differences in the rate of growth not only of the bladder, but of the entire liver and of the body region from which the liver arises. In previous communications, the most recent summary of which is Peck and Sawin ('50), it has been shown that in each of three of the races in which the distribution of gall bladder size is described above, there are different regions of greater growth.

Race X has an enlarged area adjacent to and centered on the cervicothoracic border; race V has an enlarged region extending from the level of the xiphisternum to the posterior lumbar region but centered around the thoracolumbar border in such a manner that more organs of the thoracic region manifest its influence; and race III has a center primarily involving the lumbar region, including both the thoracolumbar and lumbosacral borders and centered approximately at the 21–22nd vertebrae. Comparing this information with that in table 1 on gall bladder size in these three races, one sees that the relatively retarded growth of race X at the thoracic and lumbar regions could also explain the relatively small gall bladder of this race; and further, that the more posterior enlargement of race V could also explain a gall bladder of intermediate size; and that in race III the center of the enlarged growth area comes just below the diaphragm, where it also would be responsible for the greater average size of bladder of this race. The correlation between variations in shape of bladder and regional growth areas is by no means as obvious as in the case of size, and yet there are several bits of evidence, most of which come from interspecies observations by Boyden ('26), which indicate that such a relationship may exist.

In the first place, it should be pointed out that the range of variation observed in the rabbit, although considerable, does not cover all of the types observed in other species (see Boyden, '26). Beside the cleft or bifurcated type which is the most common type in the cat and pig, and the diverticular which is most common in cattle and sheep, two other types have been observed which do not occur in the rabbit, or if so, according to our observations, very rarely. In man, one of the most common types is the ductular bladder which is a duplicate bladder arising from a secondary outgrowth of the cystic or common bile ducts. In sheep a 4th type appears in about 1/10th of the anomalies. This is a double bladder with the accessory vesicle of trabecular origin arising from liver-cell cords on the periphery of the fossa vesicae felleae.

These usually have no connection to the primary bladder, and their position on the periphery of the fossa, where the liver parenchyma meets connective tissue, is regarded as another manifestation of the tendency of hepatic tissue to give rise to ductular or vesicular structures wherever the appropriate stimulus and space for expansion are provided.

Boyden also points out that "the most striking thing in man and in other species is the tendency toward variation in one direction." Each species has a prevailing anomalus type and one or more types which also occur repeatedly. In the cat approximately 4/5 of the anomalies are of the cleft type and 1/5 are ductular. Nearly 2/3 of those of cattle are diverticular and 1/3 are cleft. In sheep 9/10 are diverticular and 1/10 are trabecular. Swine are a little more heterogeneous but about 3/10 are cleft and 3/10 are diverticular, and in man anomalies are prevailingly ductular.

It is further significant that these species also differ in the structure of the liver. In cattle, for example, the liver is a compact mass in contrast to the many lobed organ of the cat. The non-parenchymous tissues are greatly reduced, the common duct is short and the cystic duct is long and imbedded in the liver tissue to which it is connected by minute ducts. Opportunity for expansion of aberrant portions of the duct system is therefore limited as compared with the cat, so that the distribution of anomalous vesicles in the adult is usually restricted to the area about the neck of the bladder, thus double cystic ducts are rarely seen in cattle.

Similarly, Mann et al. ('20) have found variations in the extrahepatic ducts in the rabbit, cow, sheep, dog, hog and other domestic species but no differences in relative capacity of these tracts in species without, as compared with those with, a gall bladder. Also Higgins ('26) from a comparison has concluded that other than the extent of the biliary tract and the presence or absence of the bladder there is no difference between the biliary tracts of the mouse (with) and the rat (without a bladder), except that there is a plexus of bile ducts surrounding the portal vein of the rat which may be a compensatory mechanism for concentration of bile, a function ordinarily attributed to the gall bladder.

Thus species, like races, tend to have variations of the bladder, each of its own specific kind or range. They also have variations of the form and structure of the liver, to which seem to be specifically associated certain types of anomalous gall bladders, and also variations of the ducts within and without the liver. There are also certain relationships between the concentration capacity of the liver, quantity of bile production and "sphincteric resistance," which would bear further study in races were the presence of the bladder is variable as in the rabbit.

Until recently we have made no special effort to study the variation in size, shape and superficial structure of lobation of the rabbit livers. However, the species differences (in cattle vs. cats) noted by Boyden call to mind cases of exceedingly heavy and compact livers and contrasting cases of exceedingly delicate elongated and refined lobations, which on occasion have been observed in the rabbit without recognition of their significance.

In view of the continuous quantitative series of variations in bladder size, which resembles the distribution of variations in rib and vertebrae number, the number and size of sternebrae and other variations which have been found useful as landmarks of regions of enlarged growth in the axial skeleton and adjacent structures, and in view of the nature of anomalies in bladder shape, which have been found by others to be associated with species differences in the nature of the liver and of its ducts, there seems to be good reason to suspect that these variations in both size and shape of rabbit bladders and liver may be due to the same regional growth forces.

Even though these genetic differences in the liver and gall bladder do not seem to have noticeable effects upon the well being of the animal, the underlying reasons for them merit further investigation. If the present interpretation is correct, it has considerable potential significance in so far as other organs, and particularly endocrine glands, are concerned. The fact that the liver and gall bladder stem and enlarge from a specifically localized area whose growth potential can be altered according to the genetic influences introduced by various races, suggests that a quantitative study of this region may uncover broad principles of development which may be applicable to other organs having more profound influence. If, for example, the adrenals or sex glands which contribute so much to the normal and abnormal physiological processes of the body were to be found to vary in the same way, influenced by the same or similar growth areas, it is possible that an explanation would be found for some of the irregularities in hormone imbalance which are recognized but as yet unsatisfactorily explained. Differences in size of these organs or of their parts may have a bearing upon abnormal and unorganized growth, upon resistance to disease, upon behavior patterns through the emotional state, and even, in man, upon social relations.

Although at present final proof of the theory that gall bladder and other liver variations are due to regional growth factors is lacking, there are various ways in which it can be tested. The precise antero-posterior level at which the anlage of the liver of these races arises is not known, and in so far as we know the same is true of rabbits generally. Determination of this level should also fit into the theory. On the basis of present information, if our theory is correct, we may also expect to find correlated differences in other races. The Hyde race, whose average gall bladder is large, should also have an enlarged lumbar region, including extra ribs and vertebrae, ventral spinous processes, etc.; whereas the *Ly* race with a small average bladder, which is of a mean adult body size comparable to race III, using the same means of evaluation, should have a markedly reduced lumbar region. Likewise, if the size of the gall bladder is a part of the same regional growth processes, as have been shown to be associated with other regional variations, we may expect to find the size of the liver itself influenced in the same direction. Studies designed to test these various aspects of the problem are now in progress.

SUMMARY AND CONCLUSIONS

A gall bladder is not necessarily a species characteristic of the domestic rabbit. More than 10% of the individuals in one family line of New Zealand Whites and approximately 6% in a randomly selected hybrid population of 1275 individuals lack this organ. According to size, the gall bladders of these populations and of 2205 individuals from 25 other races (some of them highly inbred) can be classified in continuous series with approximately normal frequency distributions, but varying in their range. Some races have small bladders including a complete lack of gall bladder as one extreme type, and others have large bladders including bladders as large as 103.1% of the length of the liver lobe. Racial bladder size is not correlated with body size. Evidence obtained from three sources: from comparison of racial differences in old and newborn bladders; from comparison of racial differences in bladder size with the known regional enlarged growth areas of the same races; and from study of variations in shape — all point to genetic differences in growth rate, probably of the regional type, as the cause of these variations. The importance of the gall bladder from both the functional and evolutionary standpoints and the importance of genetic studies of growth of specific organs such as the endocrine glands are discussed.

LITERATURE CITED

BOYDEN, E. A. 1926 The accessory gall bladder — an embryological and comparative study of aberrant biliary vesicles occurring in man and the domesticated animals. Am. J. Anat., *38*: 77–222.

——— 1935 The "Phrygian cap" in cholecystography. A congenital anomaly of the gall bladder. Am. J. Roentgenology, *33*: 589–602.

DEAVER, J. B., AND A. P. D. ASHHURST 1914 Surgery of the upper abdomen: 2, *l.c.*, p. 42. Blakiston.

GORHAM, F. W., AND A. C. IVY 1938 General function of the gall bladder from the evolutionary standpoint. Zool. Series Publ. 417. Field Museum Nat. History, *22:* 159–213.

HIGGINS, G. M. 1926 The biliary tract of certain rodents with and those without a gall bladder. Anat. Rec., *32:* 89–108.

MANN, F. C., S. D. BRIMHALL AND J. P. FOSTER 1920 The extrahepatic biliary tract in common domestic and laboratory animals. Anat. Rec., *18:* 47–66.

MENTZER, C. R., AND S. C. IVY 1929 Anomalous bile ducts in man. J.A.M.A., *93:* 1273–1277.

PECK. E. D., AND P. B. SAWIN 1950 Morphogenetic studies of the rabbit. VIII. Genetic variations in the sternum as determined by the interaction of general and of regionally specific growth factors. J. Exp. Zool., *114:* 335–357.

SCHMIDT, C. R., AND S. C. IVY 1937 The general function of the gall bladder. J. Cell. and Comp. Physiol., *10:* 365–385.

INDEX

ABDUCTOR pollicis longus, and extensor pollicis brevis, variations of the tendons of insertion of the 49
Adrenal cortex of the rat exposed to cold, cytological distribution of cholesterol and ascorbic acid in the 495
AMERICAN ASSOCIATION OF ANATOMISTS. Sixty-fourth annual meeting, Wayne University School of Medicine, Detroit, Michigan, March 21, 22, and 23, 1951. Proceedings, officers, and list of members 377
Amino acid deficiencies, histopathology of 313
Androgen secretion in chicken testes, role of a third gonadotrophic hormone in the mechanism of 475
ANGULO, ARMAND W. See Paff, George H. 129
Ascorbic acid in the adrenal cortex of the rat exposed to cold, cytological distribution of cholesterol and 495
Aspects of neurite outgrowth in tissue culture 145

BACCHUS, HABEEB. Cytological distribution of cholesterol and ascorbic acid in the adrenal cortex of the rat exposed to cold 495
BAKER, RICHARD F. See Pease, Daniel C. 505
BENNETT, H. STANLEY. The demonstration of thiol groups in certain tissues by means of a new colored sulfhydrol reagent 231
BHASKAR, SURINDAR NATH, ISAAC SCHOUR, EDWIN CARLETON MACDOWELL AND JOSEPH PETER WEINMANN. The skull and the dentition of screw tail mice 199
BLANDAU, RICHARD. See Odor, D. Louise 329
Bone marrow elements in foreign organs in rabbit, saponin induced colonization of the 289
Bones growing in tissue culture, zone of mineralization and the action of alizarin in preventing its appearance in 129
Brain as wet specimens in transparent plastic, mounting of stained serial slices of the 1

CAPILLARY system of the dog's spleen 65
Cat, cleavage line patterns of the 549
Chick embryo, glycogen content of various tissues of the 465
Cholesterol and ascorbic acid in the adrenal cortex of the rat exposed to cold, cytological distribution of 495
Cleavage line patterns of the cat 549
Colonization of the bone marrow elements in foreign organs in rabbits, saponin induced 289
Conical papillae of the tongue in the white rat, filiform and the 275
Cranial vault in the albino rat as measured by vital staining with alizarine red "S," growth pattern of 83
CRARY, DORCAS D. See Sawin, Paul B. 573
Cytological distribution of cholesterol and ascorbic acid in the adrenal cortex of the rat exposed to cold 495

DAWSON, ALDEN B. Histogenetic interrelationships of oocytes and follicle cells. A possible explanation of the mode of origin of certain polyovular follicles in the immature rat 181
Demonstration of the intercapillary space of the human renal glomerulus 57
Demonstration of thiol groups in certain tissues by means of a new colored sulfhydrol reagent 231
DEMPSEY, E. W. See Wislocki, G. B. 359
Dentition of screw tail mice, skull and 199
DOGGETT, THADDEUS H. The capillary system of the dog's spleen 65
Ductus arteriosus in the dog, physiological and anatomical study of the closure of the 103

EGGS, with an experimental demonstration of advantage of a method for opening and closing 41
EKSTEROWICZ, F. C. See Paff, George H. 129
Electron microscopy of nervous tissue 505
Electron microscopy of sectioned teeth 539
Electron microscopy, triple embedding for the ultrathin sectioning required by 531
Endocrine control of lymphoid tissue 17
Epithelium of mucous membranes and skin of man and monkey by the periodic acid-Schiff method, staining of stratified squamous 359
EVERETT, NEWTON B., AND ROBERT J. JOHNSON. A physiological and anatomical study of the closure of the ductus arteriosus in the dog 103
Evidences of cyclic reproductive phenomena in the rabbit 557
Extensor pollicis brevis, variations of the tendons of insertion of the abductor pollicis longus and the 49
Eyeballs in the guinea pig, weight of 349

FAWCETT, D. W. See Wislocki, G. B. 359
FELDMAN, J. D. Endocrine control of lymphoid tissue 17
FERGUSON, RALPH L. See Schwartz, Charles 313
Filiform and the conical papillae of the tongue in the white rabbit 275
Follicle cells, histogenetic interrelationships of oocytes and 181
FRANCIS, K. C. See Mulligan, R. M. 139

GARDNER, Joseph H., and Harry E. Raybuck. Cleavage line patterns of the cat ... 549
Gatz, Arthur J., and O. Boyd Houchin. Studies on the heart of vitamin E deficient rabbits ... 249
Glomerulus, demonstration of the intercapillary space of the human renal 57
Glycogen content of various tissues of the chick embryo, the ... 465
Gonadotrophic hormone in the mechanism of androgen secretion in chicken testes, role of a third ... 475
Graafian follicle, transfer function of certain cells in the wall of the ... 121
Graham, Knut. See Harkmark, Wilhelm ... 41
Graham, L. S., Jr. See McManus, J. F. A. ... 57
Growth pattern of the cranial vault in the albino rat as measured by vital staining with alizarine red "S" ... 83
Guinea pig, weight of the eyeballs in the ... 349

HAMILTON, Clara Eddy. Evidences of cyclic reproductive phenomena in the rabbit ... 557
Harkmark, Wilhelm, and Knut Graham. A method for opening and closing eggs with an experimental demonstration of its advantages ... 41
Heart of vitamin E deficient rabbits, studies on ... 249
Herniae in mammals, pericardio-peritoneal ... 113
Histogenetic interrelationships of oocytes and follicle cells. A possible explanation of the mode of origin of certain polyovular follicles in the immature rat ... 181
Histopathology of amino acid deficiencies ... 313
Hospodar, Emil W. See Kampmeier, Otto F. ... 1
Houchin, O. Boyd. See Gatz, Arthur J. ... 249

INTERCAPILLARY space of the human renal glomerulus, demonstration of ... 57

JOHNSON, Robert J. See Everett, Newton B. ... 103

KAMPMEIER, Otto F., and Emil W. Hospodar. Mounting of stained serial slices of the brain as wet specimens in transparent plastic . 1
Kutuzov, Helen, and Harry Sicher. The filiform and the conical papillae of the tongue in the white rat 275

LATIMER, Homer B. The weight of the eyeballs in the guinea pig ... 349
Lee, William H. The glycogen content of various tissues of the chick embryo ... 465
Lumsden, C. E., with the technical assistance of Mary F. Orr and Dorothy Robbins. Aspects of neurite outgrowth in tissue culture 145
Lung in the cat toward oil droplets in the circulation, reaction of the .. 267
Lupton, C. H., Jr. See McManus, J. F. A. ... 57

MACDOWELL, Edwin Carleton. See Bhaskar, Surindar Nath ... 199
Martin, A., Jr. See Wotton, R. M. .. 267
Massler, Maury, and Isaac Schour. The growth pattern of the cranial vault in the albino rat as measured by vital staining with alizarine red "S" ... 83
McManus, J. F. A., C. H. Lupton, Jr. and L. S. Graham, Jr. The demonstration of the intercapillary space of the human renal glomerulus ... 57
McShan, W. H. See Nalbandov, A. V. 475
Members, American Association of Anatomists, list of. Sixty-fourth annual meeting ... 377
Method for opening and closing eggs with an experimental demonstration of its advantages ... 41
Meyer, R. K. See Nalbandov, A. V. . 475
Mineralization and the action of alizarin in preventing its appearance in bones growing in tissue culture, zone of ... 129
Morphogenetic studies of the rabbit. X. Racial variations in the gall bladder ... 573
Mounting of stained serial slices of the brain as wet specimens in transparent plastic ... 1
Mulligan, R. M., and K. C. Francis. Weights of thyroid and parathyroid glands of normal male dogs .. 139

NALBANDOV, A. V., R. K. Meyer and W. H. McShan. The role of a third gonadotrophic hormone in the mechanism of androgen secretion in chicken testes ... 475

OBSERVATIONS on the formation of the second polar body in the rat ovum ... 329
Odor, D. Louise, and Richard Blandau. Observations on the formation of the second polar body in the rat ovum ... 329
Officers, American Association of Anatomists. Sixty-fourth annual meeting ... 377
Oil droplets in the circulation, reaction of the lung in the cat toward ... 267
Omura, Kosuke, and Bunsuke Osogue. Saponin induced colonization of the bone marrow elements in foreign organs in rabbits ... 289
Oocytes and follicle cells, histogenetic interrelationships of ... 181
Organs in rabbits, saponin induced colonization of the bone marrow elements in foreign ... 289
Osogoe, Bunsuke. See Omura, Kosuke 289
Ovum. Observations on the formation of the second polar body in the rat 329

PAFF, George H., Armand W. Angulo and F. C. Eksterowicz. The zone of mineralization and the action of alizarin in preventing its appearance in bones growing in tissue culture ... 129
Parathyroid glands of normal male dogs, weights of thyroid and ... 139
Patterns of the cat, cleavage line ... 549

Pease, Daniel C., and Richard F. Baker. Electron microscopy of nervous tissue 505
Pease, Daniel C. Electron microscopy of sectioned teeth 539
Pease, Daniel C. Triple embedding for the ultrathin sectioning required by electron microscopy 531
Pericardio-peritoneal herniae in mammals, with description of a case in the domestic cat 113
Periodic acid-Schiff method, staining of stratified squamous epithelium of mucous membranes and skin of man and monkey by the 359
Physiological and anatomical study of the closure of the ductus arteriosus in the dog 103
Proceedings, American Association of Anatomists. Sixty-fourth annual meeting 377

RABBIT, evidences of cyclic reproductive phenomena in the 557
Rabbit, Morphogenetic studies of the . 573
Rat as measured by vital staining with alizarine red "S," growth pattern of the cranial vault in the albino 83
Raybuck, Harry E. See Gardner, Joseph H. 549
Reaction of the lung in the cat toward oil droplets in the circulation . . . 267
Reed, Charles A. Pericardio-peritoneal herniae in mammals, with description of a case in the domestic cat 113
Reproductive phenomena in the rabbit, evidences of cyclic 557
Role of a third gonadotrophic hormone in the mechanism of androgen secretion in chicken testes 475

SAPONIN induced colonization of the bone marrow elements in foreign organs in rabbits 289
Sawin, Paul B., and Dorcas D. Crary. Morphogenetic studies of the rabbit. X. Racial variations in the gall bladder 573
Schour, Isaac. See Bhaskar, Surindar Nath 199
Schour, Isaac. See Massler, Maury . 83
Schwartz, Charles, Earl B. Scott and Ralph L. Ferguson. Histopathology of amino acid deficiencies. I. Phenylalanine 313
Scott, Earl B. See Schwartz, Charles 313
Second polar body in the rat ovum, observations on the formation of the 329
Sicher, Harry. See Kutuzov, Helen . 275
Sixty-fourth annual meeting, American Association of Anatomists 377
Skull and dentition of screw tail mice 199
Spleen, capillary system of the dog's . . 65
Staining of stratified squamous epithelium of mucous membranes and skin of man and monkey by the periodic acid-Schiff method 359
Stein, Arthur H., Jr. Variations of the tendons of insertion of the abductor pollicis longus and the extensor pollicis brevis 49
Studies on the heart of vitamin E deficient rabbits 249
Sulfhydrol reagent, demonstration of thiol groups in certain tissues by means of a new colored 231

TEETH, electron microscopy of sectioned 539
Thiol groups in certain tissues by means of a new colored sulfhydrol reagent, demonstration of 231
Thyroid and parathyroid glands of normal male dogs, weights of 139
Tissue culture, aspects of neurite outgrowth in 145
Tissue, electron microscopy of nervous 505
Tissue, endocrine control of lymphoid 17
Tongue in the white rat, the filiform and the conical papillae of the . . 275
Transfer function of certain cells in the wall of the Graafian follicle as revealed by their reaction to previously stained fat in the cat . . . 121
Triple embedding for the ultrathin sectioning required by electron microscopy 531

ULTRATHIN sectioning required by electron microscopy, triple embedding for the 531

VARIATIONS of the tendons of insertion of the abductor pollicis longus and the extensor pollicis brevis 49
Village, P. A. See Wotton, R. M. . . 121
Vitamin E deficient rabbits, studies on the heart of 249

WEIGHT of the eyeballs in the guinea pig 349
Weights of thyroid and parathyroid glands of normal male dogs 139
Weinmann, Joseph Peter. See Bhaskar, Surindar Nath 199
Wislocki, G. B., D. W. Fawcett and E. W. Dempsey. Staining of stratified squamous epithelium of mucous membranes and skin of man and monkey by the periodic acid-Schiff method 359
Wotton, R. M., and A. Martin, Jr. The reaction of the lung in the cat toward oil droplets in the circulation 267
Wotton, R. M., and P. A. Village. The transfer function of certain cells in the wall of the Graafian follicle as revealed by their reaction to previously stained fat in the cat 121

ZONE of mineralization and the action of alizarin in preventing its appearance in bones growing in tissue culture 129

41 A 85

COUNTWAY LIBRARY
HC 6E01